Patent and Exclusivity Search Results from query on Appl No 021549 Product 001 in the OB_Rx list.

Patent Data

Appl No	Prod No	Patent No	Patent Expiration	Drug Substance Claim	Drug Product Claim	Patent Use Code
~~021549~~	~~001~~	~~5538982~~	~~JUL 23,2013~~			
021549	001	5719147	JUN 29,2012			
021549	001	6048859	JUN 29,2012			
021549	001	6096742	JUL 01,2018			
021549	001	6235735	JUN 29,2012			

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Exclusivity Data

Appl No	Prod No	Exclusivity Code	Exclusivity Expiration
021549	001	NCE	MAR 26,2008
021549	001	I-475	OCT 28,2008

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for by soil dust and the other half by Pb mining. Sample 2p2 has a $^{206}Pb/^{207}Pb$ = 1.1827 and its age lies between 3000 (145 cm, core 2p) and 2110 ^{14}C yr BP (96 to 102 cm, core 2f). If we assume that the background soil dust signature at this time was $^{206}Pb/^{207}Pb$ = 1.1999 (Fig. 3), we can calculate the isotopic composition of the Pb ores by solving for x: 1.1827 = 0.5 (1.1999) + 0.5 (x). Using this simple approach, we calculated that the Pb ores must have had $^{206}Pb/^{207}Pb$ = 1.1655. According to J. O. Nriagu (2), mining in the Iberian Peninsula accounted for 37% of the Pb that was produced during the Iron Age (1200 to 50 B.C.), making it the most important Pb mining area of its time. Ores from these mines are known to have $^{206}Pb/^{207}Pb$ values between 1.1722 and of 1.1619 (*58*). The single most important ore body from this area is Rio Tinto, and the galenas from this ore range from $^{206}Pb/^{207}Pb$ = 1.1632 to 1.1639 [C. Pomiès, A. Cocherie, C. Guerrot, E. Marcoux, J. Lancelot, *Chem. Geol.* **144**, 137 (1998)].

35. K. J. R. Rosman, W. Chisholm, S. Hong, J.-P. Candelone, C. F. Boutron, *Environ. Sci. Technol.* **31**, 3413 (1997).
36. M. D. Davis, *Civilisations in History* (Oxford Univ. Press, Oxford, 1986).
37. M. McCormick, *Communications and Commerce: The Origins of the Early Mediterranean Economy* (Cambridge Univ. Press, Cambridge), in press.
38. Peaks in Pb concentration dating from this period are found in peat cores collected from bogs in Germany [N. Müller and N. Lamersdorf, *Telma* **25**, 143 (1995); (*9*)] and Austria (P. D. Marshall and B. S. Ottaway, in *International Symposium on the Beginning of Metallurgy*, A. Hauptmann and E. Pernicka, Eds., Bochum, Germany, 26 to 28 April 1995, in press). The most important mining areas during this period were in the Black Forest, the Harz Mountains, and the Erzgebirge; the closest important Pb mine to the bog is Sulzburg, which lies 85 km northeast of EGR; Pb mining began at this site in A.D. 1028 [H. Steuer and U. Zimmerman, *Alter Bergbau in Deutschland* (Theiss, Stuttgart, 1993)]. The single most important silver mine during this period was Rammelsberg, but it was already in decline by A.D. 1025 [H. Forshell, *The Inception of Copper Mining in Falun*, thesis and papers in Archaeology B:2, Stockholm University (1992)]; also, Rammelsberg alone is not radiogenic enough ($^{206}Pb/^{207}Pb$ = 1.168) to explain our values. The closest mine to the bog for which published Pb isotope values are available is Wiesloch with $^{206}Pb/^{207}Pb$ = 1.195 (*58*). The isotopic composition of Pb in the bog ($^{206}Pb/^{207}Pb$ = 1.1846 in the sample dating from A.D. 1020) indicates a mixture of Pb sources from medieval Pb mining in Germany.
39. J. Day and R. F. Tylecote, *The Industrial Revolution in Metals* (Institute of Metals, London, 1991).
40. D. Petit, J. P. Mennessier, L. Lamberts, *Atmos. Environ.* **18**, 1189 (1984).
41. J. R. Bacon, K. C. Jones, S. P. McGrath, A. E. Johnston, *Environ. Sci. Technol.* **30**, 2511 (1996).
42. J. G. Farmer, L. J. Eades, A. B. MacKenzie, A. Kirika, T. E. Bailey-Watts, *ibid.*, p. 3088.
43. H. C. Moor, T. Schaller, M. Sturm, *ibid.*, p. 2928.
44. D. Weiss, W. Shotyk, J. D. Kramers, P. G. Appleby, A. K. Cheburkin, in preparation.
45. Recent analyses of coals from Belgium gave $^{206}Pb/^{207}Pb$ in the range 1.171 to 1.180 [N. Walraven, B. J. H. van Os, G. Th. Klaver, J. H. Baker, S. P. Vriend, *J. Geochem. Explor.* **59**, 47 (1997)] and those from Scotland in the range of 1.184 to 1.187 [C. L. Sugden, J. G. Farmer, A. B. MacKenzie, *Environ. Geochem. Health* **15**, 59 (1993)].
46. K. Müller, thesis, University of Berne, Switzerland (1982); M. Wessels, A. Lenhard, F. Giovanoli, A. Bollhöfer, *Aquat. Sci.* **57**, 291 (1995); L. Birch, K. W. Hanselmann, R. Bachofen, *Water Res.* **30**, 679 (1996).
47. T. J. Chow and J. L. Earl, *Science* **176**, 510 (1972).
48. Swiss Federal Ministry of Environment (BUWAL) Schriftreihe Umweltschutz Nr. 256 (1995).
49. F. Monna, J. Lancelot, I. W. Croudace, A. B. Cundy, J. T. Lewis, *Environ. Sci. Technol.* **31**, 2277 (1997).
50. R. W. Hurst, T. E. Davis, B. D. Chinn, *ibid.* **30**, 304A (1996).
51. K. Olendrzynski, S. Anderberg, J. Bartnicki, J. Pacyna, W. Stigliani, *Environ. Rev.* **4**, 300 (1996).
52. F. E. Grousset *et al.*, *Environ. Sci. Technol.* **28**, 1605 (1994).
53. L. Schutz, in *Paleoclimatology and Paleometeorology: Modern and Past Patterns of Global Atmospheric Transport*, M. Leinen and M. Sarnthein, Eds., NATO ASI Series 282 (Kluwer, Dordrecht, Netherlands, 1989).
54. B. G. Andersen and H. W. Borns, *The Ice Age World* (Scandinavian Univ. Press, Oslo, Norway, 1994).
55. N. Petit-Maire, in *Continental Signals of Paleomonsoon Dynamics in Africa: Interhemispheric Perspectives*, PEP III: The Pole-Equator-Pole Transect Through Europe and Africa, F. Gasse, S. Kroepelin, F. Oldfield, Eds., PAGES Workshop Report, Series 97-2 (1997), p. 55.
56. J.-P. Candelone, S. Hong, C. Pellone, C. F. Boutron, *J. Geophys. Res.* **100**, 16605 (1995).
57. The peat core record reveals both the beginning and end of Pb pollution from German silver mining (Fig. 1) whereas the ice core record only indicates the start of this event [figure 1A of (*3*)]. Also, because the bog is much closer to the sources of anthropogenic Pb, the peat samples dating from Roman times are less radiogenic than the Greenland ice samples dating from the same period (*35*). Moreover, the results from EGR reveal not one but two distinct "preanthropogenic" aerosols: during the early Holocene, the background aerosol had Pb/Sc ~ 2 and $^{206}Pb/^{207}Pb$ = 1.205, much like average crustal rocks. However, during the middle of the Holocene, the background aerosol had Pb/Sc ~ 4, and $^{206}Pb/^{207}Pb$ = 1.199, values more typical of Saharan dust.
58. N. Grögler, J. Geiss, M. Grünenfelder, F. G. Houtermans, *Z. Naturforsch. A* **21**, 1167 (1966).
59. W. Mook and H. Streurman, *PACT* **8**, 31 (1983).
60. M. Stuiver and P. J. Reimer, *Radiocarbon* **35**, 215 (1993).
61. W.S. thanks B. Ammann, H.-P. Bärtschi, P. Blaser, C. Buck, J. Engi, L. Fonatsch, A. Fankhauser, E. Grollimund, A. Grünig, E. Hoffman, I. Immenhauser-Potthast, A. Matter, G. Ménot, C. Schlüchter, H. Seltner, P. Steinmann, J. van Leeuwen, and B. van Geel. The financial support of the Canton of Berne (SEVA Lottofonds) and the Swiss National Science Foundation (grant 20-42273.94) is sincerely appreciated. Thanks to our colleagues S. Burns, A. Hormes, T. Stocker, and two anonymous reviewers for their comments on an earlier version of this manuscript. Special thanks to B. Haas and the usual message.

11 May 1998; accepted 31 July 1998

Distinct Mechanism for Antidepressant Activity by Blockade of Central Substance P Receptors

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The localization of substance P in brain regions that coordinate stress responses and receive convergent monoaminergic innervation suggested that substance P antagonists might have psychotherapeutic properties. Like clinically used antidepressant and anxiolytic drugs, substance P antagonists suppressed isolation-induced vocalizations in guinea pigs. In a placebo-controlled trial in patients with moderate to severe major depression, robust antidepressant effects of the substance P antagonist MK-869 were consistently observed. In preclinical studies, substance P antagonists did not interact with monoamine systems in the manner seen with established antidepressant drugs. These findings suggest that substance P may play an important role in psychiatric disorders.

The development of new drugs to treat depression has been severely constrained by a poor understanding of the pathophysiology of this disease and of the mechanisms by which drugs that augment monoamine function alleviate its symptoms. The predictive validity of many preclinical assays is also limited by an inability to model psychiatric disease in animals. However, there is a pressing need for improved antidepressant therapies, given the considerable prevalence, morbidity, and mortality of depressive disorders, the incomplete efficacy of currently available drugs in many patients, and the potentially distressing adverse effects of existing therapies (*1*).

Localization of substance P in brain: Evidence for an involvement in the response to stress. Substance P is the most abundant neurokinin in the mammalian central nervous system (CNS). Mapping studies indicate that the substance P–preferring neurokinin-1 (NK_1) receptor is highly expressed

in brain regions that are critical for the regulation of affective behavior and neurochemical responses to stress (*2*). This distribution provides multiple opportunities for interactions between substance P and the convergent norepinephrine and serotonin pathways through which established antidepressant drugs act, suggesting that substance P antagonists might have utility in the treatment of psychiatric disorders. Some norepinephrine- and serotonin-containing cell bodies also coexpress substance P, presenting opportunities for more direct neuronal modulation (*2*). The potential for such functional interactions in vivo is supported by the observation that repeated administration of established antidepressant drugs causes down-regulation of substance P biosynthesis in discrete brain regions in rats, raising speculation that alterations in neurokinin systems may contribute to their antidepressant efficacy (*3*).

Activation of central substance P pathways occurs in response to noxious or aversive stimulation. Neurochemical experiments in rats revealed changes in substance P content in the hippocampus, septum, periaqueductal gray, and ventral tegmental area after inescapable foot shock, immobilization, and social isolation (*4*). Central injection of substance P or related peptide agonists induces conditioned place aversion and produces an anxiogenic profile on the elevated plus maze, implying that activation of central substance P pathways is aversive (*5*). There is, however, little direct or experimental evidence that overactivity in central substance P pathways may be involved in the pathophysiology of depression or anxiety. In one study, higher concentrations of substance P were found in the cerebrospinal fluid of depressed patients (*6*); however, this finding was not replicated (*7*).

Development and characterization of nonpeptide substance P antagonists. Since the discovery of the first nonpeptide substance P receptor antagonist, CP-96,345 (*8*), several groups have produced structurally diverse, highly selective antagonists. This created the opportunity to investigate whether selective blockade of central substance P receptors is capable of modifying responses to stress in preclinical studies (*9*). Recently, we described the synthesis of the bis(trifluoromethyl) morpholine MK-869, an orally bioavailable, long-acting substance P antagonist (*10*) that was selected for clinical development. An analog of this compound, L-760,735 (*11*), and the structurally unrelated agent L-733,060 (*12*) were used as research tools in our preclinical studies because of the availability of chemically related compounds with low affinity for the substance P receptor (L-770,765 and L-733,061, respectively) that could be used to control for nonspecific pharmacological effects. By comparing the profiles of these compounds, we were able to ensure that the effects we observed in preclinical assays were really attributable to blockade of the substance P receptor.

MK-869 and L-760,735 exhibited high affinity for the gerbil and guinea pig NK_1 receptor (mean inhibitory concentration IC_{50} = 0.3 to 0.5 nM) (*13*), and the preclinical profile of these compounds was therefore characterized using these species. The selectivity of MK-869 and L-760,735 for the human substance P receptor was much greater than for 90 other G protein–coupled receptors and ion channels (by a factor of ≥3000); no significant activities of the parent compounds or their metabolites were detected against monoamine oxidase A or B, norepinephrine, dopamine, and serotonin reuptake sites, 5-hydroxytryptamine ($5\text{-}HT_{1A}$ or $5\text{-}HT_{2A}$) receptors, monoamine transporters, or mu, delta, or kappa opiate receptors ($IC_{50} \geq 3$ μM) (*13*).

In gerbils, central infusion of substance P agonists, such as GR73632, elicits a vigorous and readily quantifiable rhythmic drumming or tapping of the hind feet, which can be inhibited by systemic administration of brain-penetrant substance P receptor antagonists (*14*). Thus, inhibition of NK_1 agonist–induced foot tapping in this species provides an in vivo functional assay for the CNS penetration of antagonists, and this enabled us to identify optimal research tools with which to investigate the role of substance P in the brain. MK-869, L-760,735, and L-733,060 all potently inhibited GR73632-induced foot tapping (mean inhibitory dose $ID_{50} \leq 0.3$ mg/kg of body weight, intravenously).

Antidepressant-like profile of substance P antagonists in preclinical assays. In guinea pigs, central infusion of substance P agonists causes locomotor activation (*15*) accompanied by pronounced and long-lasting audible vocalizations (*16*). This observation was of particular interest because psychotropic drugs that alleviate symptoms of anxiety and depression in humans are known to inhibit stress-induced vocalizations in many mammalian species (*17*). In guinea pigs, vocalizations elicited by intracerebroventricular (icv) infusion of GR73632 (0.1 nmol) were virtually abolished by pretreatment with L-733,060 (3 mg/kg), but not by its less active enantiomer L-733,061, confirming the NK_1 receptor specificity of this response. GR73632-induced vocalizations were markedly attenuated (>70%) by acute pretreatment with the antidepressant drugs imipramine and fluoxetine (30 mg/kg), but not by the anxiolytics diazepam (3 mg/kg) or buspirone (10 mg/kg) (Fig. 1). These findings show that clinically used antidepressant drugs were able to block the behavioral effects of central substance P receptor stimulation.

We then investigated the involvement of endogenous substance P release in vocalization caused by psychological stress in this species. The ability of the substance P antagonists L-760,735, L-733,060, and their low-affinity analogs to inhibit vocalizations evoked in guinea pig pups by transient maternal separation was compared with that of clinically used antidepressant and anxiolytic drugs. During 15 min of separation from their mothers and littermates, guinea pig pups emitted an audible vocalization response resembling that elicited by central infusion of GR73632. Consistent with findings in this and other species (*17*), acute administration of the antidepressant drugs phenelzine, imipramine, or fluoxetine, or of the anxiolytics diazepam (a benzodiazepine) or buspirone (a

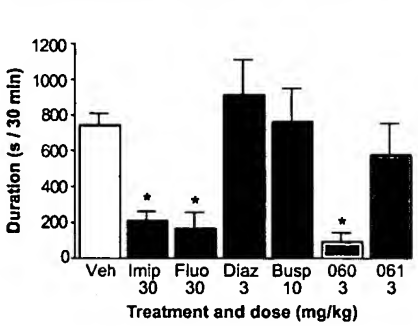

Fig. 1. Inhibition of vocalization induced by infusion of the substance P agonist GR73632 (0.1 nmol icv) in guinea pigs (*16*). Compounds were administered subcutaneously or intraperitoneally 30 min before the infusion. Imipramine (Imip), buspirone (Busp), L-733,060 (060), and L-733,061 (061) were dissolved in 0.9% saline and administered subcutaneously. Fluoxetine (Fluo) and diazepam (Diaz) were suspended in 0.5% methocel and administered intraperitoneally. Data were subjected to ANOVA followed by Dunnett's *t* test (n = 4 to 6 per group); *$p \leq 0.05$ compared with vehicle treatment.

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M. Kramer and N. Rupniak are the principal contributors to the clinical and preclinical studies, respectively.
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5-HT_{1A} receptor partial agonist), 30 min before maternal separation caused a dose-dependent and complete inhibition of separation-induced vocalizations in guinea pig pups. Similarly, the substance P antagonists L-760,735 and L-733,060 completely inhibited separation-induced vocalizations (Fig. 2). In contrast, compounds with weak NK_1 receptor affinity, L-770,765 (3 mg/kg) and L-733,061 (10 mg/kg), failed to inhibit separation-induced vocalizations (≤14%), again confirming the substance P receptor specificity of this effect. Inhibition of separation-induced vocalizations by substance P antagonists was critically dependent on their ability to penetrate the CNS because the poorly brain penetrant compounds L-743,310, LY 303870, and CGP 49823 (*14*) showed only weak activity in this assay (ID_{50} > 30 mg/kg intraperitoneally). The NK_1 receptor antagonists from our morpholine series also potently inhibited vocalizations in guinea pig pups when administered orally 4 hours before maternal separation (ID_{50} for MK-869 was 0.7 mg/kg, versus 0.9 mg/kg for L-760,735), indicating their suitability as oral therapeutic candidates. These studies demonstrate that selective pharmacological blockade of substance P receptors is capable of inhibiting behavioral responses to psychological stress in a manner resembling the effect of clinically used psychotherapeutic agents.

Demonstration that MK-869 is an efficacious and well-tolerated antidepressant in patients with major depressive disorder. A randomized double-blind placebo-controlled study was conducted to evaluate the safety and efficacy of single daily doses of

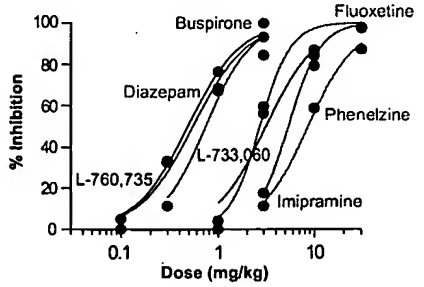

Fig. 2. Inhibition of vocalization induced by transient maternal separation of guinea pig pups. Pups were prescreened to ensure that a vocalization response was reproducibly elicited after maternal separation. Pups were placed individually in a room isolated from the home cage for 15 min, and the duration of vocalization was recorded. Animals vocalizing for ≥5 min were used for drug challenge studies. Each pup received a subcutaneous or intraperitoneal injection of test compound (as described for Fig. 1) and was returned to the home cage for 30 min before maternal separation, as described above. The duration of vocalization on the drug treatment day is expressed as a percentage of the pretreatment baseline value for each animal (n = 4 to 6).

300 mg of MK-869 in comparison to paroxetine (20 mg) or placebo in outpatients with major depressive disorder (MDD) and moderately high anxiety. MK-869 was chosen to test the concept clinically because of its high affinity, selectivity, brain penetrance, duration, and oral bioavailability that permitted a once daily oral dosing regimen. MK-869 was well tolerated in human volunteer studies at 300 mg, a dose for which pharmacokinetic data predicted >90% blockade of central substance P receptors.

The study was conducted at four experienced investigative sites (*18*). Eligible patients completed a washout of previous psychotropic medications (*19*) and were randomized in equal numbers to receive MK-869, paroxetine, or placebo (*20*). Efficacy measurements were made at the end of weeks 1, 2, 4, and 6, or on termination. The primary efficacy outcome measure was the 21-item Hamilton depression (HAM-D21) total score; secondary measures included the Hamilton anxiety (HAM-A) total score and the Clinical Global Impressions severity scale (CGI-S) (*21*). The demographics of the patients in the three treatment groups were comparable (*22*).

The principal outcome was a 4.3-point difference in mean change from baseline to week 6 between MK-869 and placebo in total HAM-D21 score, confirming that MK-869 is an efficacious antidepressant (Fig. 3A). The effect of MK-869 was similar to that of

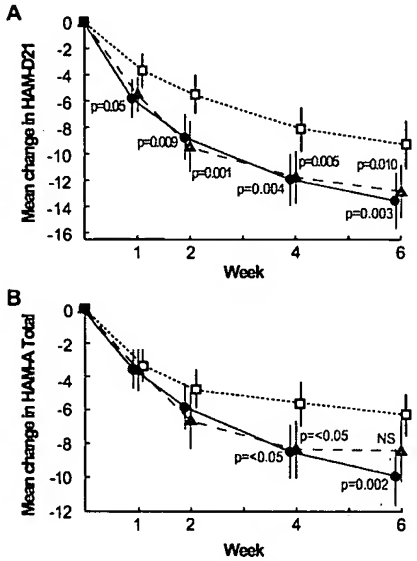

Fig. 3. Effect of treatment with MK-869 (300 mg/day) or paroxetine (20 mg/day) on mean change from baseline on the Hamilton Depression Scale (HAM-D21) (A) and the Hamilton Anxiety Scale (14 items) (B) in patients with major depressive disorder. Comparisons are of MK-869 (red circles, n = 66) or paroxetine (green triangles, n = 68) versus placebo (open squares, n = 64). Error bars show 95% confidence intervals.

paroxetine (mean change of 3.6 points). The antidepressant effect of MK-869 was observed at all four investigative sites [differences of 4.1, 6.6, 3.3, and 3.2 points, respectively (*22*)] and was corroborated by all secondary measures. A standard measure of response, ≥50% change from baseline to week 6 in total HAM-D21 score, was compared for the different treatment groups. Of the patients receiving MK-869, 54% showed improvements of ≥50% from baseline, compared with 46% for paroxetine and 28% in the placebo group. In addition, 43% of patients treated with MK-869 reached scores of <10 points (generally considered to be a complete response) on the HAM-D17 at last rating, compared with 33% of patients treated with paroxetine and 17% of patients receiving placebo.

Mean changes from baseline to week 6 for the four factors of the HAM-D21 showed that the antidepressant profiles of MK-869 and paroxetine were generally similar (*23*). Changes for each of the 21 items of the HAM-D21 were explored further to compare the profile of MK-869 with that of paroxetine. Both drugs were superior to placebo on many items of the HAM-D21. Patients receiving MK-869 showed more improvement than those on paroxetine on items of insomnia (early; item 4) and genital symptoms (item 14), whereas paroxetine showed more improvement than MK-869 on item 17, insight. MK-869 also demonstrated significant anxiolytic activity in this population of depressed patients. An anxiolytic effect was gradually observed, which continued to increase through week 6 (Fig. 3B).

The safety and tolerability of MK-869 were generally similar to placebo, except for mild and typically transient somnolence and asthenia, side effects also observed with paroxetine (Table 1). The most common clinical adverse experiences (AEs) observed in patients receiving MK-869 were headache (32%), somnolence (20%), nausea (18%), and asthenia/fatigue (14%); these were generally mild and transient. Nausea, which occurred in 29% of patients on paroxetine compared with 10% of those on placebo, was the chief AE causing discontinuation of treatment with paroxetine. Notably, the incidence of sexual dysfunction in patients receiving paroxetine (a problem observed with other serotonin reuptake inhibitors) was 26%, significantly greater than with MK-869 (3%) or placebo (4%). In addition, discontinuation as a result of clinical AEs was more frequent among patients receiving paroxetine (19%) than among patients receiving MK-869 (9%) or placebo (9%). There was no pattern in the types of clinical AEs that caused discontinuation in patients on MK-869 or placebo. There were no reports of drug-seeking behavior (symptoms of drug withdrawal or any other AEs suggestive of a potential for drug abuse) or clinically signif-

icant changes in vital signs, physical examination, weight, or electrocardiograms in patients treated with MK-869 (*24*).

Novel mechanism of antidepressant activity. These findings provide clinical evidence that substance P antagonism represents a well-tolerated, distinct mechanism for antidepressant activity. The antidepressant effect of MK-869 was observed at all investigative sites and was consistently corroborated by all secondary measures—that is, HAM-D items (for example, item 1, depression), most HAM-D factors, percentages of patients achieving ≥50% response, percentages of patients with HAM-D scores of <10, and clinical global ratings. Because paroxetine showed a similar effect (mean change of 3.6 points) on the HAM-D21, the study had good assay sensitivity. In addition, the results of the "as observed" and "last observation carried forward" analyses were similar, indicating that the results were not markedly affected by carrying forward data in patients who discontinued (*25*). MK-869 also demonstrated significant anxiolytic activity in this population of depressed patients.

Anxiety levels of the depressed patients in this study were moderately high but within the range expected for a population with a primary diagnosis of depression. The differential time course of the antidepressant and anxiolytic effects (seen from visual inspection of the curves), and the similar profiles of MK-869 and paroxetine on the HAM-D21 factors and items, suggest that the antidepressant effects of MK-869 are independent of its potential anxiolytic effects.

An important question raised by these findings is whether substance P antagonists and established antidepressant drugs really act via distinct molecular targets (for example, monoamine transporters or the NK_1 receptor). We investigated whether markers of monoamine function were changed after acute administration of the substance P antagonist L-760,735 in vivo. In gerbils, treatment with reserpine caused hypothermia and ptosis. Consistent with the results of previous studies using mice (*26*), phenelzine and imipramine reversed these effects, whereas fluoxetine and L-760,735 did not (all test compounds administered at 30 mg/kg). Moreover, repeated administration of a substance P antagonist for 14 days did not cause downregulation of cortical β-adrenoreceptors in rats (*27*). These findings show that central substance P receptor blockade does not augment norepinephrine function in a manner resembling the action of monoamine oxidase inhibitors or tricyclic antidepressants.

The ability of L-760,735 to potentiate 5-HT–mediated behaviors (*28*) was compared with that of other antidepressant drugs in gerbils. Administration of the 5-HT precursor 5-hydroxytryptophan in animals pretreated with pargyline (100 mg/kg intraperitoneally) caused a behavioral syndrome comprising wet dog shakes, forepaw treading, splaying of the hindlimbs, and flattened body posture. The frequency or number of animals exhibiting these behaviors was increased by administration of monoamine reuptake inhibitors as compared with animals receiving vehicle, but not in animals treated with L-760,735 (all compounds tested at 30 mg/kg). Consistent with these findings, acute central substance P receptor blockade did not affect extracellular 5-HT concentration, as measured by in vivo microdialysis in the rat hippocampus (*29*).

Thus, L-760,735 did not augment norepinephrine or serotonin function in the manner seen with established antidepressant drugs. The atypical profile of L-760,735 in these assays supports the proposal that the antidepressant activity of substance P antagonists is mediated via a novel mechanism, as was also reflected by the absence of sexual dysfunction, nausea, or other adverse effects associated with established antidepressant drugs in patients. Because many clinically used antidepressant drugs have relatively poor pharmacological specificity, the possibility that their therapeutic effects might be explained through a direct blockade of central substance P receptors was examined. A range of structurally diverse antidepressant drugs (phenelzine, imipramine, fluoxetine, mianserin, reboxetine) was found to have no significant affinity for human, guinea pig, and gerbil NK_1 receptors (IC_{50} > 10 μM) (*13*).

Possible CNS sites for antidepressant activity of substance P antagonists. There are many potential CNS sites that might mediate the antidepressant activity of substance P antagonists and other classes of antidepressant drugs. Of these, the amygdala in particular has been implicated as a potential site for the action of established antidepressant drugs. Thus, focal injection of imipramine into the amygdala produces effects in assays involving psychological stress resembling those seen after systemic administration (*30*).

A major output projection from the amygdala is to the hypothalamus. In cats, electrical stimulation of the amygdala facilitates the emergence of a defensive rage syndrome elicited by stimulation of the hypothalamus. Imipramine and related antidepressants block attack behavior caused by hypothalamic stimulation in cats (*31*). Substance P provides a powerful monosynaptic input from the medial amygdala to the hypothalamus that is important for the expression of defensive rage in cats. Thus, both systemic and intrahypothalamic infusion of the substance P antagonist CP-96,345 blocks the facilitatory effects of amygdaloid stimulation on defensive rage (*32*). A second major projection from the amygdala is to the periaqueductal gray (PAG), where electrical stimulation also causes defensive rage behavior in cats. The PAG shows dense immunoreactivity for substance P, and the amount of preprotachykinin mRNA has been shown to increase in the dorsal PAG after social defeat stress in rats (*33*). These and other substance P–containing pathways may therefore be important in the response to stressors, particularly in relation to vocalization responses examined in the present studies using guinea pigs.

After activation, substance P NK_1 receptors are internalized and at least 1 hour elapses before the protein is recycled into the neuronal membrane (*34*). We exploited this internalization as a way to map those brain regions in which release of endogenous substance P occurred after maternal separation of guinea pig pups (*35*). We saw an increase (about 60%) in the number of cells showing NK_1 receptor internalization in the anterior-basolateral amygdala after maternal separation for 5 min (*36*) (Fig. 4). We conclude that psychological stress causes release of sub-

Table 1. Incidence (≥5%) of clinical adverse experiences (AEs) in patients receiving MK-869 (300 mg) or paroxetine (20 mg). Only those AEs observed at rates numerically greater than with placebo are shown.

AE	Percentage of patients		
	MK-869 (n = 71)	Paroxetine (n = 72)	Placebo (n = 70)
Nervous system and psychiatric			
Headache	32	28	24
Somnolence	20	19	9
Insomnia	11	14	9
Irritability	7	1	0
Nervousness	1	6	4
Digestive			
Nausea	18	29*	10
Diarrhea	11	15	9
Dry mouth	9	8	7
Flatulence	7	3	4
Dizziness	7	8	6
Anorexia	4	11	3
Respiratory			
Upper respiratory infection	6	8	3
Skin and appendages			
Sweating	3	11	3
Urogenital			
Total sexual dysfunction: combined terms	3†	26*	4
Libido decreased	0	6	0
General sexual dysfunction	0†	8*	0
Ejaculation disorder (% males)	3†	20	7
Impotence (% males)	3	10	4
General			
Asthenia/fatigue	14	19*	4
Abdominal pain	9	6	3

*Paroxetine greater than placebo, $p \leq 0.05$. †MK-869 less than paroxetine, $p \leq 0.05$.

stance P in the amygdala. It is not yet known whether stress-induced NK_1 receptor internalization is inhibited by substance P antagonists; however, this possibility is strongly suggested by the prevention of substance P–induced receptor internalization in the striatum of rats by the NK_1 receptor antagonist RP 67580 (*34*).

Conclusions. MK-869, a brain-penetrant substance P antagonist, represents an innovative mechanistic approach to antidepressant therapy. The precise mechanism by which these therapeutic effects are brought about is not yet known, as is the case for traditional antidepressant therapies, but preclinical evidence suggests that it may involve the integration of emotional responses to stress by brain structures such as the amygdala. The possibility that alterations in substance P or the NK_1 receptor are primarily involved in the pathogenesis of depression requires further investigation, which may lead to a better understanding of the pathophysiology of this disease.

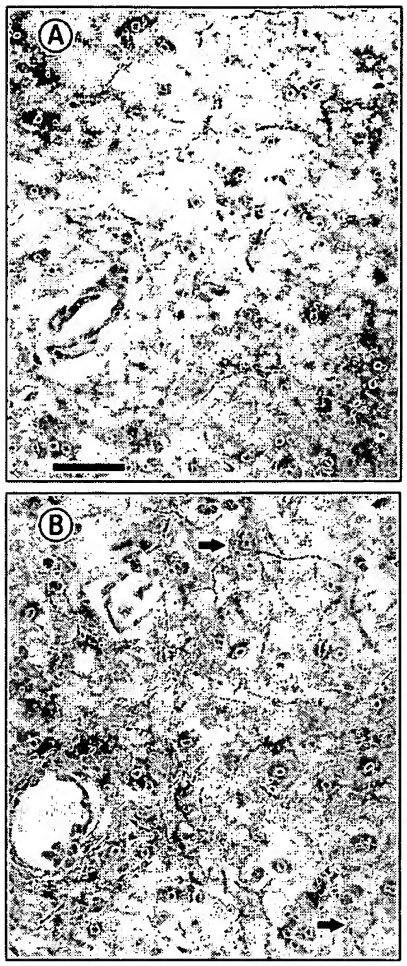

Fig. 4. Immunocytochemical demonstration of NK_1 receptor endocytosis in anterior-basolateral amygdala in response to maternal separation of guinea pig pups. **(A)** In nonisolated guinea pig pups, NK_1 receptor immunoreactivity (IR) is associated with the somatic and dendritic surfaces of the neurons. **(B)** After maternal separation for 5 min, there was a marked transfer of NK_1 receptor IR from the cell surface to intracellular endosomes, accompanied by structural reorganization of dendrites characterized by varicosities rich in NK_1 receptor–positive endosomes and linked by thin fibers (arrows). Scale bar, 50 μM. ANOVA revealed an increase ($p \leq 0.016$) in the number of cells in the anterior-basolateral amygdala exhibiting NK_1 receptor endocytosis in animals separated from their mothers compared with nonisolated pups ($n = 5$ or 6).

References and Notes

1. *MIMS Annual* (Tien Wah Press, Singapore, ed. 20, 1996), pp. 3.250–3.268; *Martindale the Extra Pharmacopoeia* (Royal Pharmaceutical Society, London, ed. 21, 1996), pp. 299–340; *Physicians' Desk Reference* (Medical Economics Company, Montvale, NJ, ed. 51, 1997), pp. 2494–2509.
2. P. W. Mantyh, S. P. Hunt, J. E. Maggio, *Brain Res.* **307**, 147 (1984); T. Hokfelt *et al.*, in *Psychopharmacology: The Third Generation of Progress*, H. Y. Meltzer, Ed. (Raven, New York, 1987), pp. 401–416; E. P. Pioro, J. M. Mai, A. C. Cuello, in *The Human Nervous System*, G. Paxinos, Ed. (Academic Press, New York, 1990), pp. 1051–1094.
3. N. Barden *et al.*, *J. Neurochem.* **41**, 834 (1983); E. Brodin, S. O. Ogren, E. Theodorsson-Norheim, *Neuropharmacology* **26**, 581 (1987); L. A. Riley, R. P. Hart, G. M. Jonakait, *Mol. Cell. Neurosci.* **2**, 139 (1991); Y. Shirayama *et al.*, *Brain Res.* **739**, 70 (1996).
4. A. Lisoprawski, G. Blanc, J. Glowinski, *Neurosci. Lett.* **25**, 47 (1981); R. A. Siegel *et al.*, *Neurochem. Int.* **6**, 783 (1984); M. J. Bannon *et al.*, *Brain Res.* **381**, 393 (1986); A. Rosen *et al.*, *Acta Physiol. Scand.* **146**, 341 (1992); E. Brodin *et al.*, *Neuropeptides* **26**, 253 (1994).
5. P. J. Elliott, *Exp. Brain. Res.* **73**, 345 (1988); R. M. Teixeira *et al.*, *Eur. J. Pharmacol.* **311**, 7 (1996); M. S. Aguiar and M. L. Brandao, *Physiol. Behav.* **60**, 1183 (1996).
6. R. Rimon *et al.*, *Biol. Psychiatry* **19**, 509 (1983).
7. W. H. Berrettini *et al.*, *ibid.* **20**, 965 (1985).
8. R. M. Snider *et al.*, *Science* **251**, 435 (1991).
9. This was complicated by interspecies variants in neurokinin receptor distribution [M. M. Dietl and J. M. Palacios, *Brain Res.* **593**, 211 (1991)] and pharmacology [B. D. Gitter *et al.*, *Eur. J. Pharmacol.* **197**, 237 (1991); I. J. M. Beresford *et al.*, *Br. J. Pharmacol.* **104**, 292 (1991)], by nonspecific pharmacological effects of antagonists [A. Nagahisa *et al.*, *ibid.* **107**, 273 (1992); N. M. J. Rupniak *et al.*, *ibid.* **110**, 1607 (1993)], and by the poor CNS penetration of many compounds [see (*14*)].
10. J. J. Hale *et al.*, *J. Med. Chem.*, in press.
11. L-760,735 and L-770,765 were synthesized as described in *International (PCT) Publication WO 95/18124* (1995).
12. T. Harrison *et al.*, *Bioorg. Med. Chem. Lett.* **4**, 2545 (1994); N. M. J. Rupniak *et al.*, *Pain* **67**, 189 (1996).
13. Affinity for the cloned human, guinea pig, and rat NK_1 receptor expressed in intact CHO or COS cells was determined as described [M. A. Cascieri *et al.*, *Mol. Pharmacol.* **42**, 458 (1992)]. Affinity for the gerbil NK_1 receptor was similarly determined using synaptosomal membranes (50 μg) purified from brain cortex as described [M. A. Cascieri, G. G. Chicchi, T. M. Ling, *J. Biol. Chem.* **260**, 1501 (1985)]. See *Science* Online (www.sciencemag.org) for NK_1 receptor affinity data for MK-869, L-760,735, L-770,765, L-733,060, and L-733,061. Affinity for human NK_2 and NK_3 receptors was determined after expression in CHO cells as described [S. Sadowski *et al.*, *Neuropeptides* **24**, 317 (1993)]. Affinity for more than 90 other neurotransmitter receptors and ion channels was determined by Panlabs DiscoveryScreen; assays for monoamine transporters were based on P. Berger *et al.*, *Eur. J. Pharmacol.* **177**, 91 (1990); A. M. Cesura *et al.*, *ibid.* **186**, 95 (1990); S. M. Tejani-Butt *et al.*, *ibid.* **191**, 239 (1990); J. W. Boja *et al.*, *Synapse* **12**, 27 (1992).
14. N. M. J. Rupniak and A. R. Williams, *Eur. J. Pharmacol.* **265**, 179 (1994); L. J. Bristow and L. Young, *ibid.* **254**, 245 (1994); E. A. Graham, M. P. Turpin, C. M. Stubbs, *Neuropeptides* **24**, 228 (1993); A. Vassout *et al.*, *ibid.* **26**, 538 (1994); N. M. J. Rupniak *et al.*, *Eur. J. Pharmacol.* **326**, 201 (1997).
15. P. J. Brent, P. A. Johnston, L. A. Chahl, *Neuropharmacology* **27**, 743 (1988); P. A. Johnston and L. A. Chahl, *Arch. Pharmacol.* **343**, 283 (1991); G. S. Mason, C. A. Graham, P. J. Elliott, *Br. J. Pharmacol.* **105**, 251P (1992); N. M. J. Rupniak and A. Jackson, *Eur. J. Pharmacol.* **262**, 171 (1994).
16. Dunkin-Hartley guinea pigs (250 to 300 g) were anesthetized and a 27-gauge needle was inserted into the cerebral ventricles, 7.5 to 8 mm below bregma. GR73632 was injected icv (5 μl) and spontaneous behavior was recorded upon recovery from anesthesia ($n = 4$ to 6).
17. J. P. Scott, *Excerpta Med. Int. Congr. Ser.* **359**, 735 (1974); J. Panksepp *et al.*, *Biol. Psychiatry* **13**, 607 (1978); B. Herman and J. Panksepp, *Pharmacol. Biochem. Behav.* **9**, 213 (1978); *Science* **211**, 1060 (1981); R. D. Porsolt, S. Roux, M. Jalfre, *Pharmacol. Biochem. Behav.* **20**, 979 (1983); T. R. Insel, J. L. Hill, R. B. Mayor, *ibid.* **24**, 1263 (1986); N. H. Kalin, S. E. Shelton, C. M. Barksdale, *Brain Res.* **408**, 192 (1987); J. D. Newman, J. T. Winslow, D. L. Murphy, *ibid.* **538**, 24 (1991); K. A. Miczek *et al.*, *Psychopharmacology* **121**, 38 (1995); H. E. Molewijk *et al.*, *ibid.* **128**, 31 (1996).
18. The study protocol was approved by investigational review boards. Patients gave informed consent to the requirements and restrictions of the study. Male and female depressed outpatients in good physical health, ages 18 to 65, were eligible. Females were required to use adequate contraceptive methods during the study. Patients were excluded if they were considered to be at risk for suicide or violence. Patients with ≥25% decrease on the HAM-D17 between the screening and end-of-washout evaluations were disqualified from entry. Patients were required to have DSM-IV diagnosis of MDD [single or recurrent; American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders* (Washington, DC, ed. 4, 1994)], a current episode of ≥4 weeks but <2 years in duration; and the following scores at screening and end-of-washout visits: ≥22 (moderately depressed) on the 17-item version of the HAM-D, ≥15 (moderately high anxiety) on the HAM-A scale, and ≥4 (moderately ill) on the CGI scale [*Early Clinical Drug Evaluation Unit (ECDEU) Assessment Manual* (Department of Health, Education and Welfare Publication ADM 76-338, 1976), pp. 583–585]. Safety observations included physical examination, weight, laboratory evaluations, vital signs, and electrocardiogram and AE reporting.
19. The drug washout period was 7 ± 3 days except for fluoxetine (4 weeks) and monoamine oxidase inhibitors (2 weeks). Chloral hydrate (500 to 1000 mg daily) could be prescribed sparingly for insomnia during the study, but this was not to be taken within 24 hours before clinic visits. All other psychotropic medications were prohibited.
20. All medications were to be taken orally, once daily in the evening, for 6 weeks. MK-869 (three 100-mg tablets per dose, with matching placebo capsules to paroxetine) and encapsulated paroxetine 20-mg tablets (with matching placebo to MK-869) were used in a double-dummy design. Paroxetine tablets were placed in opaque capsules to maintain the blind; these had comparable stability and dissolution to the unencapsulated form.
21. The primary efficacy analysis compared mean changes from baseline between MK-869 and placebo on HAM-D21 total score at week 6 using an "all patients treated, last observation carried forward" approach. For a two-tailed test with $p = 0.05$, the power to detect a 4-point difference of change (a clinically significant effect) between MK-869 and placebo was 84% (based on standard deviation of 8.0). Pairwise comparisons of MK-869

or paroxetine versus placebo were performed at each week by analysis of variance (ANOVA) with treatment group and investigative site included as factors. Statistical testing at early time points was performed post hoc and was not adjusted for multiplicity. Similar analyses evaluated secondary efficacy variables. Pairwise comparisons of clinical AEs, or discontinuations for AEs, used Fisher's exact test.

22. Seventy-one patients were treated with MK-869 (300 mg/day), 72 with paroxetine (20 mg/day), and 70 with placebo. About 70% of the patients in each group completed the full 6-week course of therapy. The demonstration of antidepressant efficacy of MK-869 was not confounded by different rates of discontinuation in the treatment groups. The treatment × site effect was significant (ANOVA, $p = 0.031$). The interactions were not qualitative between any two treatment groups. Therefore, the ANOVA model with treatment and site was applied for treatment comparisons. See *Science* Online (www.sciencemag.org) for details on demographics and baseline comparability, an accounting of patients who completed or discontinued from the study, and statistics on interaction of treatment × investigative site (with respect to mean change from baseline to week 6 in HAM-D21 total scores).
23. See *Science* Online (www.sciencemag.org) for details on mean changes from baseline in Hamilton Depression Scale factors with MK-869, paroxetine, and placebo, with pairwise comparison p values for MK-869 and paroxetine versus placebo.
24. Mild transaminase elevations (1.5 to 2.5 times the upper limit of normal) were observed in three patients receiving MK-869 and caused discontinuation. High titers of Epstein-Barr antibodies (immunoglobulin G) and clinical signs of viral infection were observed in two of these patients; in the third, AST was mildly elevated on entry into the study. One patient was discontinued on paroxetine for elevated transaminases. Mild transient increases in transaminases (AST, ALT, or both) were regarded as laboratory AEs in two additional patients on MK-869, four on paroxetine, and one on placebo.
25. See *Science* Online (www.sciencemag.org) for details on mean changes from baseline in HAM-D21 data ("as observed" approach) with MK-869, paroxetine, and placebo, with pairwise comparison p values for MK-869 and paroxetine versus placebo.
26. M. Bourin *et al.*, *Arzneim. Forsch. Drug Res.* **33**, 1173 (1983); J. Maj *et al.*, *Pol. J. Pharmacol. Pharm.* **35**, 49 (1983).
27. GR 205171, a compound with high affinity for the rat NK_1 receptor [C. J. Gardner *et al.*, *Regul. Pept.* **65**, 45 (1996)], was administered by osmotic mini-pump (3 mg/kg per day) for 14 days and did not down-regulate cortical β-adrenoceptors in rats (P. H. Hutson, unpublished data).
28. S. J. Peroutka, R. M. Lebovitz, S. H. Snyder, *Science* **212**, 827 (1981); B. F. Koe *et al.*, *J. Pharmacol. Exp. Ther.* **226**, 686 (1983).
29. GR 205171 did not affect 5-HT efflux in the hippocampus when administered to rats at 10 mg/kg, as measured using in vivo microdialysis [P. H. Hutson, unpublished data; methods as described by P. H. Hutson *et al.*, *Neuropharmacology* **34**, 383 (1995)].
30. Z. P. Horovitz *et al.*, *Int. J. Neuropharmacol.* **5**, 405 (1966); G. E. Duncan *et al.*, *J. Pharmacol. Exp. Ther.* **238**, 758 (1986).
31. B. Dubinsky and M. E. Goldberg, *Neuropharmacology* **10**, 537 (1971).
32. M. B. Shaikh, A. Steinberg, A. Siegel, *Brain Res.* **625**, 283 (1993).
33. E. M. Nikulina *et al.*, *Soc. Neurosci. Abstr.* **23**, 126 (1997).
34. P. W. Mantyh *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2622 (1995); A. M. Garland *et al.*, *Mol. Pharmacol.* **49**, 438 (1996).
35. Guinea pig pups were either left in their family group (control) or isolated for 5 min before terminal anesthesia and perfusion. Sections from the amygdala were subjected to antigen retrieval [S. Shi *et al.*, *J. Histochem. Cytochem.* **39**, 741 (1991)]. NK_1 receptor immunoreactivity was examined with an antibody supplied by R. Shigemoto [R. Shigemoto *et al.*, *Neurosci. Lett.* **153**, 157 (1993)] using standard indirect enzymatic detection techniques.
36. Serial sections of the anterior-basolateral amygdala were assessed for endocytic morphology. Neurons displaying NK_1 receptor–positive endosomes were counted in three consecutive sections to establish mean cell counts.
37. We thank D. Slaughter, C. Assang, M. Beer, S. Patel, C. Barton, P. H. Hutson, F. Kelleher, M. Rigby, S. Boyce, and R. Shigemoto for their contributions to the preclinical studies.

31 March 1998; accepted 28 July 1998

ELSEVIER

European Journal of Pharmacology 412 (2001) 255–264

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Inhibition of shock-induced foot tapping behaviour in the gerbil by a tachykinin NK_1 receptor antagonist

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Received 18 December 2000; accepted 22 December 2000

Abstract

The selective tachykinin NK_1 receptor antagonist, 2-(*R*)-(1-(*R*)-3,5-Bis(trifluoromethyl)phenylethoxy)-3-(*S*)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine (MK-869), has been recently described as a novel therapeutic approach for anxiety/depression. A frequently used model to establish the central nervous system (CNS) activity of tachykinin NK_1 receptor antagonists is the inhibition of NK_1 agonist-induced foot tapping in gerbils. In the present study, we demonstrate that foot tapping can also be induced in most, but not all, gerbils by footshock and associated cues. MK-869 (0.3–3 mg/kg, i.p.) dose-dependently blocked this foot tapping response. This effect was further shown to be due to selective NK_1 receptor blockade, since (2*S*,3*S*)-*cis*-3(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994; 3 mg/kg, i.p.) inhibited foot tapping, whereas its less active enantiomer (2*R*,3*R*)-*cis*-3(2-methoxybenzylamino)-2-phenylpiperidine (CP-100,263; 3 mg/kg, i.p.) had no effect. Diazepam (1–10 mg/kg, i.p.) also inhibited foot tapping, whereas fluoxetine (10–30 mg/kg, i.p.) markedly increased this behaviour. The present data support the view that foot tapping in the gerbil is a behavioural response to an aversive stimulus, and is robustly inhibited by two NK_1 receptor antagonists. The data support a role for tachykinin NK_1 receptor antagonists as novel anxiolytic/antidepressants.

Keywords: Tachykinin NK_1 receptor antagonist; Foot tapping; (Gerbil); Fear conditioning; Anxiety; Depression

1. Introduction

Following the recent publication of Kramer et al. (1998), there has been considerable interest in Neurokinin (NK)1 receptor antagonists as novel treatments for anxiety and depression. These workers reported that in a 6-week double-blind, placebo-controlled study in patients with major depression, the selective NK_1 receptor antagonist, 2-(*R*)-(1-(*R*)-3,5-Bis(trifluoromethyl)phenylethoxy)-3-(*S*)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine (MK-869) (300 mg/day), produced a positive outcome as measured by both the Hamilton depression (HAM-D21) and anxiety (HAM-A) scales. The effects were equivalent to that of the selective serotonergic reuptake inhibitor, paroxetine (20 mg/day), and side effects typically associated with this drug class, e.g. nausea, sexual dysfunction were diminished in the MK-869 group. Taken together, the study of Kramer et al. (1998) may represent an important advance in the search for novel treatments of anxiety and depression (Nutt, 1998).

In gerbils, a species whose NK_1 receptor pharmacology resembles that of the human (Gitter et al., 1991; Beresford et al., 1991), the intracerebroventricular (i.c.v.) administration of the NK_1 agonist, GR73632 (D-Ala [L-Pro9, Met-Leu10]-substance P-(7–11)), produces a characteristic rhythmic tapping of the hind feet (Graham et al., 1993). This robust and readily quantifiable response is inhibited by brain-penetrating antagonists of the NK_1 receptor (Graham et al., 1993; Rupniak and Williams, 1994; Bristow and Young, 1994). Consequently, NK_1 agonist-induced foot tapping in the gerbil has become a valuable in vivo assay for the identification of centrally acting tachykinin NK_1 receptor antagonists (Rupniak et al., 1997; Hale et al., 1998). Since foot tapping in the gerbil has also been reported following electroshock or offset of reward it is postulated to be a species specific response to an aversive stimulus (Routtenberg and Kramis, 1967). Given

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)00724-5

the clinical evidence that NK_1 receptor antagonists may have antidepressant/anxiolytic properties (Kramer et al., 1998), and the robust nature of foot tapping behaviour following NK_1 agonist administration, we sought to investigate ways of non-pharmacologically inducing this behaviour in gerbils.

In the present study, we have used a Pavlovian fear-conditioning procedure. In a variety of species, the pairing of specific cues (conditioned stimulus) with an aversive stimulus (unconditioned stimulus), typically electroshock, subsequently results in the conditioned stimulus inducing a variety of fear related behaviours (see LeDoux, 1998; Davis, 1999 for reviews). In rodents, this typically involves freezing or increased startle responses, as well as autonomic signs such as defaecation, increased heart rate and arterial blood pressure (LeDoux et al., 1988; Davis, 1999). Following the establishment of suitable shock parameters in gerbils, we studied their behaviour both during the conditioning session and also in a retest session, performed 24 h later where the animals are presented with the conditioned stimulus in the absence of the unconditioned stimulus. We found that foottapping behaviour could be induced in some, but not all, gerbils during both the conditioning and retest session. Consequently, we studied the effect of the tachykinin NK_1 receptor antagonists MK-869 (Hale et al., 1998) and (2*S*,3*S*)-*cis*-3(2-methoxybenzylamino)-2-phenyl piperidine (CP-99,994) (McLean et al., 1993), as well as a clinically efficacious anxiolytic (diazepam) and antidepressant (fluoxetine) against this shock-induced foot tapping. Some of this work has been published in abstract form (Ballard et al., 1999).

2. Materials and methods

2.1. Subjects

Male and female Mongolian gerbils (Biological Research Laboratories, Füllinsdorf, Switzerland and Charles River, USA), weighing between 40 and 70 g, were used in all experiments. Gerbils were housed four per cage with food and water available ad libitum, in temperature and humidity-controlled holding rooms. The animals were allowed 4–7 days to acclimatize to the housing conditions prior to testing. All testing was conducted during the light phase of the light/dark cycle (lights on: 0600–1800 h). Experimentally naive gerbils were used in each study. All experiments were carried out under the guidelines issued under local Cantonal and Swiss federal law.

2.2. Shock-induced foot tapping

The test apparatus consisted of a Perspex chamber ($14 \times 14 \times 13$ cm [$L \times W \times H$], Med Associates, USA) with a grid floor through which a scrambled electrical stimulus could be applied. A cue light was located on one wall, and a sonalert system on the ceiling, which was capable of delivering a 2900-Hz tone. Two visually distinct chambers were used, one of white perspex, the other black perspex. In all other aspects, the chambers were essentially identical. Gerbils were run in the same chamber during both the conditioning and retest session. Also, the chamber type was balanced across all treatment groups. The delivery of electroshock and the presentation of the light and tone conditioned stimulus was controlled by Med PC (Med Associates, USA).

Preliminary experiments suggested that shock levels between 1 and 2 mA were required to induce responses such as flinch, vocalisation and jump in gerbils. At levels below 1 mA, no consistent response to footshock was observed. Consequently, an experimental protocol was designed whereby a 2-min initial familiarisation period was followed by 6×1 s footshocks delivered at 60-s intervals. The onset of each electrical stimulus was preceded by a 30-s light/tone conditioned stimulus. Three shock intensities were tested: 0 mA ($n = 11$); 1 mA ($n = 11$); 2 mA ($n = 13$). The total duration of the conditioning session was 8 min. In a retest session performed 24 h later, the animals were reexposed to the conditioning box for a 3-min period followed by three presentations of the 30-s conditioned stimulus separated by 30-s intervals. Thus, the total duration of the retest session was 6 min. At no time was footshock delivered during this retest session. The time spent on foot tapping and the immobility time (i.e., freezing) were recorded at each 30-s time bin, and the number of faecal boli were counted at the end of each session. Immobility time was operationally defined as total immobility of the animal except for respiratory movement.

In experiments to study the effect of drug treatment on shock-induced foot tapping, gerbils were pretreated prior to placement into the conditioning box where they were subjected to 6×1 s electrical stimuli (2 mA) timed at 60-s intervals. Each footshock was signalled by presentation of a 30-s light/tone cue (conditioned stimulus). MK-869 ($n = 14$–26 per group)-treated gerbils were retested 24 h later (without drug administration). CP-99,994 ($n = 10$–12 per group), diazepam ($n = 9$–10 per group; $n = 4$ at 10 mg/kg) and fluoxetine ($n = 10$–12 per group) were only studied on the conditioning session. To determine whether drug treatment altered shock perception, the percentage of animals displaying each of the following responses: flinch, vocalisation and jump, at least once during the session was calculated.

During the course of these experiments, a small proportion of gerbils developed seizures on placement into the test chamber. These animals were always excluded from further study. Differences in group sizes usually reflect this adjustment.

2.3. Tachykinin NK_1 agonist-induced foot tapping

Gerbils were anaesthetised by inhalation of isoflurane/oxygen mixture, the scalp was exposed and GR73632

(0.3–10.0 pmol/5 μl) or vehicle (0.1% bovine serum albumin) was administered into the lateral ventricles (i.c.v.) via a cuffed 25-gauge needle vertically inserted to a depth of 4.5 mm below bregma. The incision was closed using a clip suture and gerbils were placed into perspex boxes on recovery of their righting reflex. Duration of foot tapping behaviour over a 5-min period was measured.

2.4. Drugs and injections

MK-869, CP-99,994, CP-100,263 ((2*R*,3*R*)-*cis*-3(2-methoxybenzylamino)-2-phenylpiperidine), GR73632, diazepam and fluoxetine were all synthesised within the Chemistry department at F. Hoffmann-La Roche (Basel). All test substances were dissolved in 0.3% Tween 80 v/v 0.9% NaCl and administered in an injection volume of 10 ml/kg intraperitoneally (i.p.) 30 min prior to testing. GR73632 was dissolved in 0.1% bovine serum albumin in water and frozen (−20°C) in aliquots until use, where it was given by direct intracerebroventricular injection.

2.5. Statistical analysis

One-factor Analysis of Variance (ANOVA) followed, in significant cases, by Fisher's Least Significant Difference test was used to analyse the immobility time and

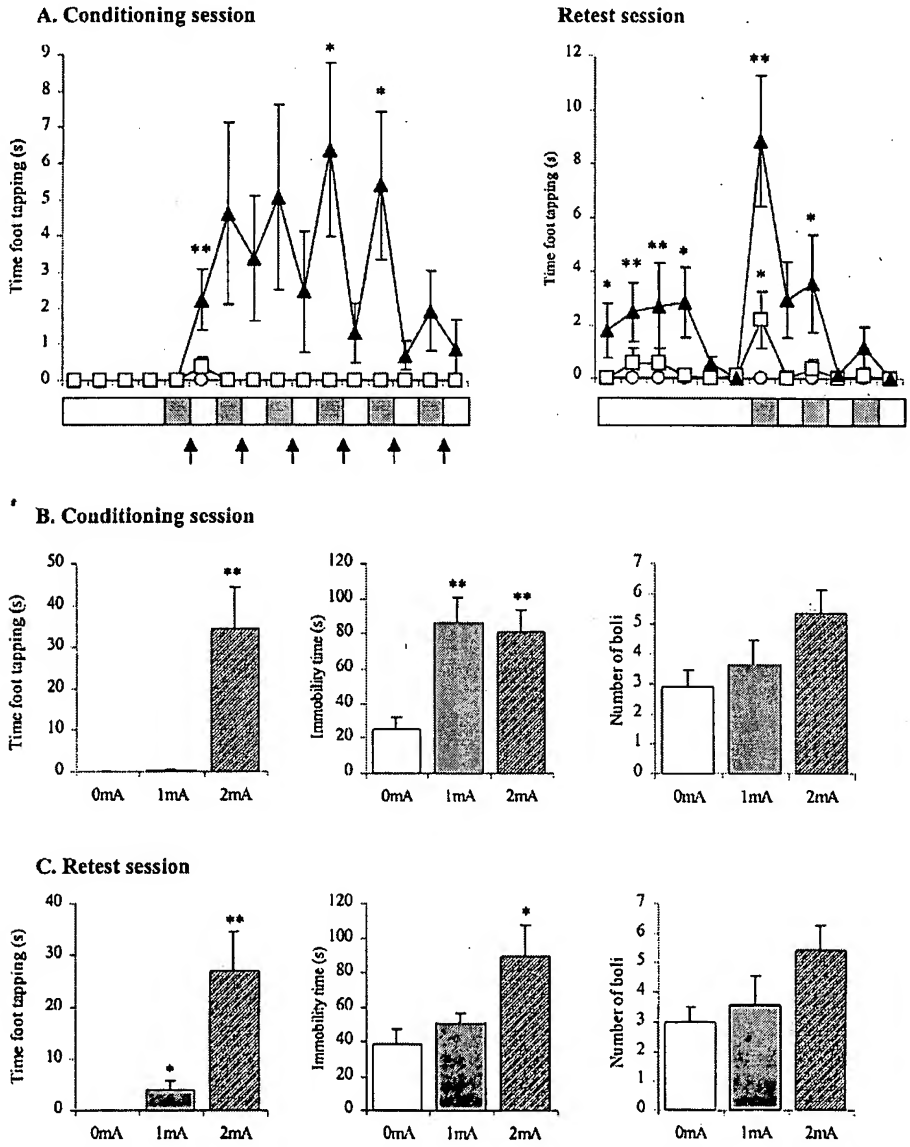

Fig. 1. Characterisation of various behaviors in gerbils during a fear-conditioning procedure and during a retest session performed 24 h later where the gerbils were presented with cues previously paired with footshock presentation (see Materials and methods for further details). (A) Temporal distribution of foot tapping behaviour recorded at 30-s time bins over the conditioning and retest session. ○ = No shock controls, □ = 1 mA intensity, ▲ = 2 mA intensity. The shaded area along the *x*-axis indicates the conditioned stimulus (light + tone) presentation, the vertical arrows indicate shock presentation (unconditioned stimulus). Data collapsed for foot tapping, freezing and defaecation over (B) the conditioning, and (C) the retest session are also shown. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 0 mA vehicle controls.

defaecation scores. Since foot tapping was not observed in all gerbils, and marked inter-animal differences were found in those that did respond, this measure was analysed by Kruskal–Wallis test followed by post hoc Mann Whitney *U*-test. Correlation coefficients comparing: (1) immobility time and foot tapping scores in the conditioning and retest session; and (2) foot tapping scores in the conditioning and retest sessions, were calculated for shocked controls in the characterisation of shock-induced foot tapping experiment. All statistical analysis was conducted using StatView for Windows (Version 5.0.1, SAS Institute).

3. Results

3.1. Characterisation of shock-induced foot tapping in gerbils

Following placement in the test chambers, over the initial 2-min (unshocked) period, all gerbils showed reasonable activity with minimal freezing and no foot tapping behaviour. However, following the first conditioned stimulus–shock pairing, there was a marked incidence of foot tapping in the 2 mA, but not in the control (no shock) or 1 mA group. Although in percentage terms, the mean amount of time engaged in this behaviour was relatively small (6–20%), it was maintained over successive conditioned stimulus–shock pairings, reaching significance during the fourth and fifth conditioned stimulus presentation (Fig. 1A), but by the sixth conditioned stimulus–shock pairing the incidence of foot tapping was in decline. During the course of this experiment, it was evident that not all gerbils within the 2-mA group demonstrated reliable foot tapping behaviour—the actual proportion being 85%. Inclusion of responders and non-responders yielded an overall score collapsed over the entire 8-min session of 34 ± 10 s (range: 0–97 s; Fig. 1B). Immobility time was indistinguishable between groups over the initial 2 min, although following the first conditioned stimulus–shock pairing its incidence increased in both the 1- and 2-mA groups, which were similar, although both were significantly different to the control (unshocked) group ($F(2,32) = 7.6$, $P < 0.01$) (Fig. 1B). Although a modest increase in defaecation was recorded in the 2-mA group, this narrowly failed to reach significance ($F(2,32) = 3.2$, $P = 0.06$) (Fig. 1B). Comparison between immobility time and foot tapping scores in the 2-mA group yielded a correlation of borderline significance (correlation coefficient -0.56, $P = 0.04$), suggesting a trend toward higher immobility scores in gerbils having lower foot tapping scores.

In the retest session, some foot tapping was evident during the initial 3-min period in the 2-mA group in which no conditioned stimulus presentations were made. This was presumably a response to contextual cues associated with the foot shock (Phillips and LeDoux, 1992). However, the first light/tone conditioned stimulus presentation produced a marked foot tapping response following both 1 and 2 mA footshock, but again not in all gerbils (Fig. 1A). There was a significant correlation (correlation coefficient $+0.82$, $P < 0.01$) between foot tapping scores recorded during the conditioning and retest session (Fig. 2). A main effect on immobility time was also found in the retest experiment ($F(2,32) = 4.1$, $P < 0.05$), although this measure was only increased in the 2-mA group (Fig. 1C). A comparison between immobility time and time spent foot tapping in the 2-mA group again revealed a trend toward higher immobility scores in gerbils having lower foot tapping scores, however this correlation did not reach significance (correlation coefficient -0.51, $P = 0.07$).

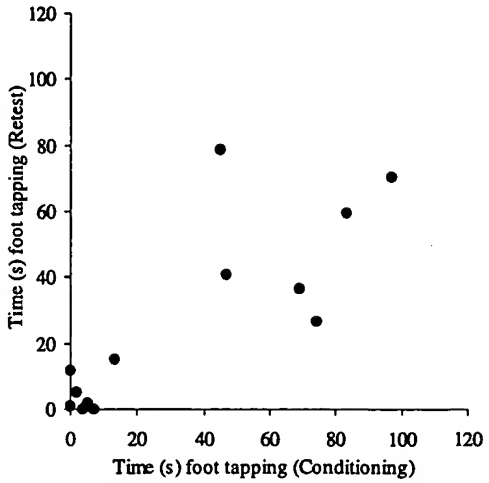

Fig. 2. Correlation between time spent foot tapping in the conditioning session compared to time spent foot tapping in the retest session for gerbils taken from the 2-mA group in experiment 1.

3.2. Effect of the tachykinin NK_1 receptor antagonists MK-869 and CP-99,994 against shock-induced foot tapping

Pretreatment with MK-869 (0.3–3 mg/kg) produced a significant reduction in foot tapping ($H = 23.3$, $DF = 4$, $P < 0.01$) induced by a 2-mA electrical stimulus (Fig. 3A). In this experiment, no overall main effect on the immobility time was found ($F(4,89) = 1.5$, $P = 0.2$), despite the fact that the time engaged in this behaviour appeared to be higher in shocked compared to unshocked controls (Table 1). An overall main effect of defaecation ($F(4,85) = 4.8$, $P < 0.01$) was recorded, due to the difference between shocked and unshocked controls (Table 1). MK-869 did not reduce the shock-induced increase in this measure. MK-869 did not affect shock perception, since all gerbils displayed both vocalisation and flinch response and the majority of animals produced a jump response to the shock (Table 2). In the retest session (Fig. 3B), gerbils that were treated with MK-869 prior to the conditioning session

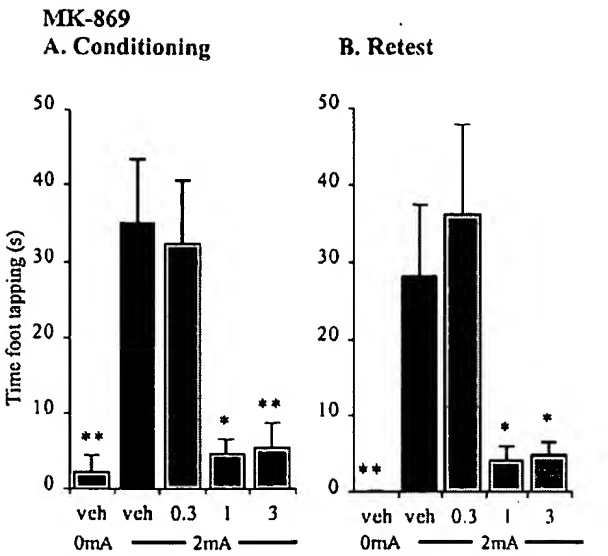

Fig. 3. Effect of MK-869 ($n = 14$–26 per group) on gerbil foot tapping (A) induced by a 2-mA electrical stimulus during the conditioning session and (B) in response to the conditioned stimulus during the retest session. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 2 mA vehicle controls.

Table 2
Percentage of gerbils displaying flinch, vocalisation or jump in response to footshock

Treatment	Dose (mg/kg)	Flinch	Vocalisation	Jump
MK-869	Vehicle (2 mA)	100	100	69
	0.3	100	100	53
	1	100	100	80
	3	100	100	70
CP-99,994	Vehicle (2 mA)	100	100	50
	3	100	100	46
	CP-100,263	100	100	64
Diazepam	Vehicle (2 mA)	100	100	100
	1	100	100	50
	3	100	100	0
	10	100	100	0
Fluoxetine	Vehicle (2 mA)	100	100	17
	3	100	100	42
	10	100	100	33
	30	90	100	33

produced a significant inhibition of the foot tapping response ($H = 22.9$, $df = 4$, $P < 0.001$). There was no overall main effect on the immobility time ($F(4,89) = 2.3$, $P = 0.07$) (Table 1). MK-869 also did not affect the shock-induced increase in defaecation (Table 1).

CP-99,994 (3 mg/kg) similarly reduced shock-induced foot tapping ($H = 19.7$, $df = 3$, $P < 0.01$), while its less active enantiomer CP-100,263 (3 mg/kg) was inactive

Table 1
Immobility time and number of faecal boli during conditioning and retest sessions

Treatment	Dose (mg/kg)	Conditioning: immobile time (s)	Conditioning: no. of faecal boli	Retest: immobile time (s)	Retest: no. of faecal boli
MK-869	Vehicle (0 mA)	23 ± 5	4 ± 1[a]	30 ± 9	2 ± 1[b]
	Vehicle (2 mA)	42 ± 5	7 ± 1	54 ± 8	6 ± 1
	0.3	42 ± 9	7 ± 1	59 ± 12	7 ± 1
	1	35 ± 4	6 ± 1	30 ± 4	6 ± 1
	3	45 ± 7	7 ± 1	63 ± 12	5 ± 1
CP-99,994	Vehicle (0 mA)	39 ± 11	2 ± 1	NT	NT
	Vehicle (2 mA)	37 ± 7	5 ± 1		
	3	59 ± 15	5 ± 1		
	CP-100,263	59 ± 12	5 ± 1		
Diazepam	Vehicle (0 mA)	28 ± 10	1 ± 1[b]	NT	NT
	Vehicle (2 mA)	45 ± 8	5 ± 1		
	1	55 ± 7	5 ± 1		
	3	205 ± 23[b]	2 ± 1[b]		
	10	261 ± 18[b]	1 ± 1[b]		
Fluoxetine	Vehicle (0 mA)	15 ± 11[c]	5 ± 1	NT	NT
	Vehicle (2 mA)	52 ± 12	7 ± 1		
	3	29 ± 15	6 ± 1		
	10	3 ± 3[a]	6 ± 1		
	30	15 ± 11[c]	4 ± 1		

[a] $P < 0.01$ vs. vehicle (2 mA) group (ANOVA followed by post hoc Fisher's PLSD test).
[b] $P < 0.001$ vs. vehicle (2 mA) group (ANOVA followed by post hoc Fisher's PLSD test).
[c] $P < 0.05$ vs. vehicle (2 mA) group (ANOVA followed by post hoc Fisher's PLSD test).

(Fig. 4A). No overall main effect was found on measures of immobility time ($F(3,39) = 1.0$, $P = 0.4$) and defaecation ($F(3,39) = 2.5$, $P = 0.07$). In this experiment, the control (unshocked) baseline immobility time was relatively high (Table 1). Shock perception was not altered following pretreatment with either CP-99,994 or CP-100,263. All the gerbils tested had a flinch and vocalisation response to the footshock, the jump response was more variable but unrelated to drug pretreatment (Table 2).

3.3. Effect of diazepam and fluoxetine against shock-induced foot tapping

Diazepam (1–10 mg/kg) produced a highly robust inhibition of shock-induced foot tapping ($H = 27.1$, $df = 4$, $P < 0.01$), with the effect at the 1 mg/kg dose being of borderline significance ($P = 0.06$) and complete inhibition at the 3 and 10 mg/kg doses (Fig. 4B). A significant main effect was also found for immobility time ($F(4,38) = 43.0$, $P < 0.01$) and defaecation ($F(4,38) = 9.3$, $P < 0.01$) in this experiment. Although no increase in immobility time was observed for the shocked vs. unshocked group, there was a highly significant increase in this measure in gerbils pretreated with diazepam at the 3 and 10 mg/kg doses, which probably reflects a diazepam-induced reduction in general activity (Table 1). The inhibition of foot tapping was not due to muscle relaxation since diazepam did not significantly affect grip strength (vehicle: 152 ± 23 g ($n = 5$); 3 mg/kg: 130 ± 7 g ($n = 6$);10 mg/kg: 106 ± 14 g ($n = 6$); ANOVA $F(3,19) = 1.6$, $P = 0.2$). Defaecation was significantly reduced relative to shocked controls at these doses. Diazepam treatment did not alter the vocalisation and flinch response to the shock, although at 1 mg/kg the jump response was reduced and at 3–10 mg/kg this response was absent (Table 2).

In contrast to diazepam, fluoxetine (10–30 mg/kg) actually increased the duration of foot tapping relative to shocked controls (Fig. 4C). Indeed, this effect was highly significant, with gerbils recording up to 292 s foot tapping during the test period ($H = 30.0$, $df = 4$, $P < 0.0001$). Fluoxetine also reduced the immobility time ($F(4,50) = 2.9$, $P < 0.05$), this being likely due to the fact that the gerbils under fluoxetine appeared to engage in more active behaviours such as foot tapping. In this experiment, there was no main effect of defaecation ($F(4,50) = 1.3$, $P = 0.3$). Shock perception was unaltered by fluoxetine, since the flinch, vocalisation and jump response did not differ from the vehicle group (Table 2).

3.4. Effect of test compounds against tachykinin NK_1 receptor agonist-induced foot tapping

Intracerebroventricular injection of the NK_1 agonist, GR73632, produced a dose-related incidence of foot tapping throughout the 5-min test session. Indeed at the 3 and 10 pmol doses, the gerbils foot tapped virtually throughout the entire observation period (Fig. 5A), having cumulative scores of 275 ± 8 and 284 ± 4 s at the 3 and 10 pmol doses, respectively. This behaviour was dose-dependently blocked by pretreatment with the tachykinin NK_1 receptor antagonists MK-869 (0.3–3 mg/kg; Fig. 5B) and CP-99,994 (3–10 mg/kg), but not by CP-100,263 (3–10

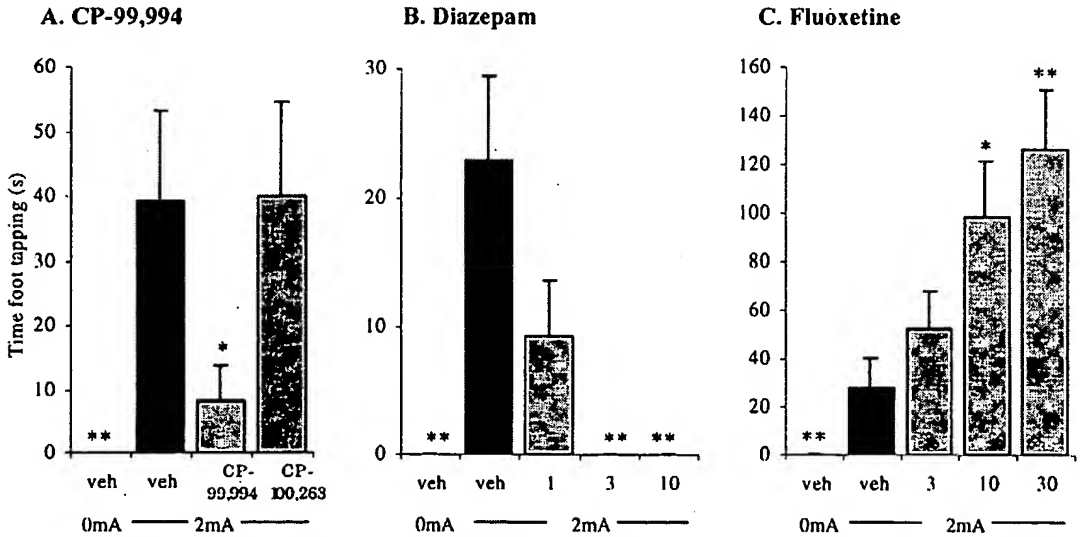

Fig. 4. Effect of (A) CP-99,994 and CP-100,263 ($n = 10$–12 per group); (B) diazepam ($n = 9$–10 per group; $n = 4$ at 10 mg/kg); (C) fluoxetine ($n = 10$–12 per group) on gerbil foot tapping induced by a 2-mA electrical stimulus. Note the different y-axis scales. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 2 mA vehicle controls.

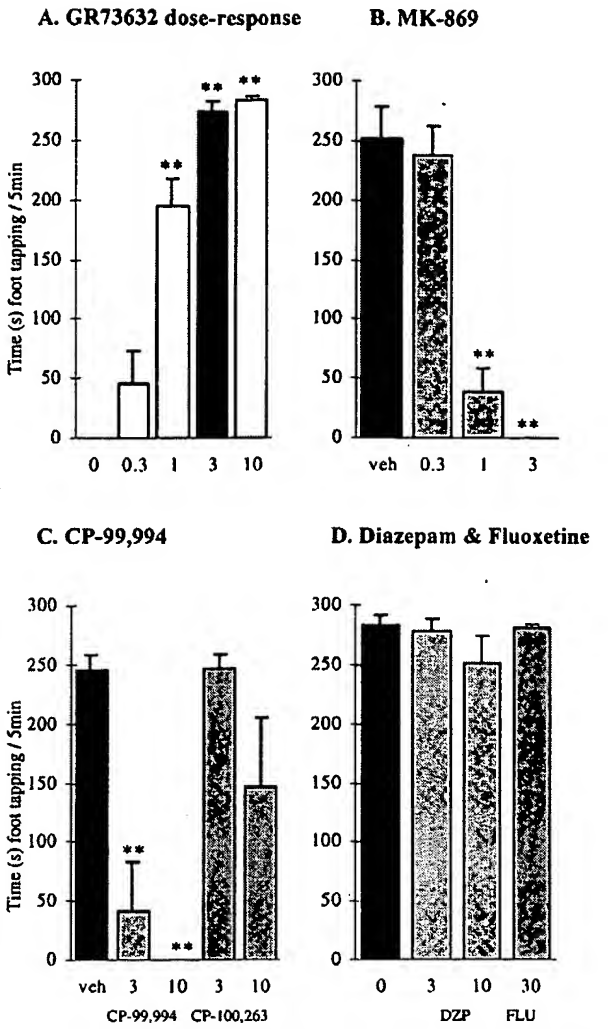

Fig. 5. (A) Dose response for GR73632-induced foot tapping in gerbils. The 3 pmol/5 μl i.c.v. dose was subsequently chosen for antagonist studies. Effect of the tachykinin NK_1 receptor antagonists (B) MK-869, and (C) CP-99,994 against GR73632-induced foot tapping. Note that the less active isomer CP-100,263 was only weakly active in this test. (D) Effect of diazepam (3 and 10 mg/kg) and fluoxetine (30 mg/kg) against GR73632-induced foot tapping ($n = 5–9$ per group). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle pretreated controls.

mg/kg) (Fig. 5C). Neither diazepam (3–10 mg/kg) nor fluoxetine (30 mg/kg) pretreatment affected the foot tapping response produced by GR73632 (Fig. 5D).

4. Discussion

In the present study, we have demonstrated that foot tapping may be elicited in gerbils by an aversive stimulus, i.e. electroshock, and notably by cues paired with this unconditioned stimulus. Such fear-conditioning procedures have been used to induce fear in rodents (LeDoux, 1998; Davis, 1999), the present data suggests foot tapping may be an expression of fear and/or anxiety in the gerbil. Evidence for a neurokinin involvement in this behaviour is supported by the finding that the tachykinin NK_1 receptor antagonists MK869 and CP-99,994 completely block shock-induced foot tapping. Measurement of responsivity to footshock during conditioning, suggested that the effect of both tachykinin NK_1 receptor antagonists was not due to changes in shock perception.

There is clinical evidence that tachykinin NK_1 receptor antagonists produce anxiolytic effects (Kramer et al., 1998), however in preclinical studies this evidence has been limited by the prevalence of tests utilising rats and mice, whose NK_1 receptor pharmacology differs to that of the human receptor (Gitter et al., 1991; Beresford et al., 1991). Nonetheless, the tachykinin NK_1 receptor antagonist (±)-CP-96,345, has been reported to increase the time spent in the more aversive light compartment in a mouse light/dark test, albeit at sedative doses (Zernig et al., 1992). File (1997) also reported anxiolytic activity of the tachykinin NK_1 receptor antagonist, CGP 49823 in a rat social interaction test. Furthermore, NK_1 receptor activation has been shown to induce anxiogenesis in mice, an effect which was inhibited by antagonists at this receptor (Texeira et al., 1996). However, in order to characterise these compounds effectively in animal models of anxiety and depression, it is preferable to develop tests using animal species with a similar NK_1 receptor pharmacology to human, such as gerbils and guinea pigs.

It was for this reason that we examined the present gerbil fear conditioning test. In the initial behavioural characterisation, we also identified increased immobility time (i.e. freezing behaviour) and defaecation during both the conditioning and retest sessions. Indeed immobility time appeared to be the most sensitive measure, since it was significantly increased in the 1-mA group, whilst foot tapping was only evident in the 2-mA group. However, in subsequent experiments, shock-induced changes in this measure became variable, due perhaps to baseline differences seen across studies. Since we attempted no discrimination between immobility and freezing, it was operationally defined as complete cessation of movement except respiratory (e.g., see Phillips and LeDoux, 1992), a more detailed ethological approach to scoring this and other fear related behaviours may be appropriate (e.g., horizontal tail shaking, eye lid closure). Similarly defaecation in subsequent tests gave inconsistent results. Thus, foot tapping emerged as the most robust response—it was rarely seen in unshocked controls (total 2%), and the incidence was reasonably consistent across experiments.

However, in contrast to the NK_1 agonist-induced foot tapping, only 80–85% of the gerbils actually did foot tap following electroshock, and to variable degrees. Hendrie and Starkey (1998) have reported that only male gerbils displayed foot-tapping behaviour in a social interaction test. Yet in the present study, the animals sex did not seem to contribute to the variance. The robustness of this response might be improved by pre-selecting gerbils based

on their foot tap response to another noxious environmental stimulus, however as yet we have not explored this approach. In addition, it is possible that housing conditions may alter this behavioural response (see Clark and Galef, 1979; Hendrie and Starkey, 1998). The reasonable correlation between individual foot tapping scores recorded in the conditioning and test sessions, certainly suggest that individual gerbils differ in their propensity to demonstrate this behaviour. This positive correlation has also been replicated in subsequent experiments. In addition, the comparison between freezing and foot tapping scores in the 2-mA group indicated a trend toward higher freezing/immobility scores in gerbils having lower foot tapping scores. This suggests that these are mutually exclusive behaviours for the expression of fear and/or anxiety, i.e. if an animal is engaged in an 'active' behaviour such as foot tapping, then it is unable to express a 'passive' behaviour such as freezing. Foot tapping has also been shown to occur in other situations, e.g. mating, indicating that this behaviour may not only be an expression of fear, but also of heightened arousal. However, within the present fear conditioning procedure gerbils foot tapped in response to the various cues predictive of footshock, as well as to the footshock itself. This would imply that in the present experimental paradigm, foot tapping is a behavioural response to an aversive stimulus, and a likely index of fear and/or anxiety.

Examination of the temporal distribution of foot tapping during the conditioning session, revealed that this behaviour was more evident during the conditioned stimulus presentation, compared to the intervening time period. Interestingly, rather than a gain in intensity with repeated shock-conditioned stimulus pairings, by the end of the session it was in decline. Whether this reflects a behavioural adaptation or perhaps receptor desensitization (NK_1 receptor internalisation?) is unclear, and may be worthy of further study. Indeed, Smith et al. (1999) reported immunocytochemical evidence for NK_1 receptor endocytosis within the basolateral amygdala following immobilisation stress in gerbils. Since the amygdala is a critical neuroanatomical locus for the formation and storage of information processes relating to mammalian fear conditioning (see LeDoux, 1998; Davis, 1999; Maren, 1999 for recent reviews), one might predict similar changes in this procedure. Indeed, stress-, including shock-induced changes in substance P content have been reported in diverse regions of the central nervous system (CNS) (Bannon et al., 1986; Brodin et al., 1994; Hahn and Bannon, 1999). Furthermore, there is evidence for a substance P projection pathway from the medial amygdaloid nucleus to the medial hypothalamus, which seems to be involved in the expression of defensive rage behaviour (Shaik et al., 1993).

The selective, brain penetrant NK_1 receptor antagonists MK-869 and CP-99,994, but not its less active enantiomer CP-100,263 (McLean et al., 1993; Tattersall et al., 1993), blocked shock-induced foot tapping. This effect occurred at doses that produced no signs of ataxia or myorelaxation. The doses that blocked shock-induced foot tapping were similar to those that blocked the pharmacologically mediated response. Taken together, these data strongly support a role for NK_1 receptors in the mediation of shock-induced foot tapping. Pharmacological dissociations between the inhibition of shock and NK_1 agonist-induced foot tapping were seen with diazepam, which failed to reduce the latter response. This finding essentially eliminates a sedative action of diazepam to account for the antagonism seen in the shock-induced model, as the gerbils treated at these doses of benzodiazepine were clearly capable of emitting this behaviour. Also muscle relaxation was not evident at these doses, since grip strength measures were similar between diazepam-pretreated gerbils and controls. Since the dose of GR73632 (3 pmol/5 μl) was selected to produce a near maximal foot tapping response, it may be less amenable to attenuation by non-tachykinin NK_1 receptor antagonists. We are presently looking at lower doses of GR73632 (0.5–1 pmol/5 μl) to see if various anxiolytic/antidepressant drugs will affect this response. Interestingly, GR73632-induced vocalisations in guinea-pigs are attenuated by some (but not all) drugs belonging to this class, e.g. imipramine, fluoxetine, but not diazepam. In contrast, vocalisations in guinea-pig pups induced non-pharmacologically by maternal separation seem to be reliably blocked by a wide range of anxiolytics and antidepressant drugs, including diazepam (Kramer et al., 1998; Rupniak et al., 2000).

There was some preliminary evidence for dissociations between the effects of diazepam and the tachykinin NK_1 receptor antagonists on shock-induced defaecation. Diazepam robustly inhibited both shock-induced foot tapping and defaecation, whereas the tachykinin NK_1 receptor antagonists only blocked the foot tapping response. This might suggest that tachykinin NK_1 receptor antagonists only block certain fear-related behaviours, and perhaps not somatic signs, however, a tachykinin NK_1 receptor antagonist has been shown to decrease restraint stress-induced defaecation in the rat (Ikeda et al., 1995) and so further work is necessary to establish the generality of this result. The finding that diazepam increased immobility time was a surprise observation and seems contrary to the known anxiolytic effects of benzodiazepines. However, we feel that this likely reflects a limitation to the present technique for scoring this behaviour and does not reflect diazepam-induced increased freezing scores. Rather, it may reflect that diazepam pretreatment reduced some spontaneous behaviours in this test. Since we attempted no discrimination between measuring immobility and freezing, as discussed earlier, a more detailed ethological approach to scoring gerbil behaviour in this test may be necessary.

NK_1 receptors have been proposed to play a role in the modulation of nociception, and indeed CP-99,994 has been shown to produce an analgesic effect in mice, particularly

against the late phase of formalin-induced licking (Seguin et al., 1995). Therefore, it is possible that the tachykinin NK_1 receptor antagonists produced an analgesic effect such that the gerbils had a reduced perception of the shock. However, in all experiments, tachykinin NK_1 receptor antagonist-pretreated gerbils displayed responses to footshock that were identical to controls. Moreover, CP-99,994 has been shown to be more effective against prolonged noxious chemical stimuli and only at ataxia-producing doses does CP-99,994 block the reflexive response to mechanical and thermal noxious stimuli (Seguin et al., 1995). In addition, NK_1 receptor knockout mice do not differ in their acute nociceptive thresholds when compared to wild-type mice, which suggests that substance P does not mediate acute pain sensation (De Felipe et al., 1998).

A further potential confound to the interpretation that tachykinin NK_1 receptor antagonists reduce shock-induced foot tapping by a reduction of fear/anxiety, is that these drugs may impair the learning processes essential to form associations between the shock unconditioned stimulus and the conditioned stimulus. However, to the best of our knowledge there is no evidence to suggest that tachykinin NK_1 receptor antagonists impair learning. For instance, NK_1 knockout mice can form conditioned associations between food or an acute cocaine injection and a novel environment (Murtra et al., 2000). However, one way to empirically test this is to study the effect of tachykinin NK_1 receptor antagonists on foot tapping induced by cue reexposure (retest) after conditioning in a drug-free state. In the present study, MK869-pretreated gerbils were retested 24 h after conditioning and a similar blockade of the foot tapping response was recorded. However, this drug does have a long duration of action (Hale et al., 1998), so it is possible that the gerbils were not completely drug-free at retest.

Fluoxetine was found in these studies to actually increase foot tapping induced by footshock. This may be related to the anxiogenesis frequently reported following acute pretreatment with selective serotonin reuptake inhibitors (Van Praag, 1988; Westenberg and Den Boer, 1988; Bodnoff et al., 1989; Griebel et al., 1995). It would be interesting to establish whether this apparent potentiation remains following chronic fluoxetine treatment, for tolerance tends to develop to the acute anxiogenic effects of selective serotonin reuptake inhibitors (Bodnoff et al., 1989; Griebel et al., 1994, 1995). It would also be of value to study other anxiogenic compounds on gerbil foot tapping behaviour.

In conclusion, the present series of experiments suggest a novel approach for looking at NK_1 receptor antagonists on gerbil behaviour. An advantage of this test is that it utilises a species whose NK_1 receptor pharmacology resembles human. As yet we have only studied the effect of drugs on behaviour during the conditioning session—we have not systematically studied the effects of tachykinin NK_1 receptor antagonists on the foot tapping produced by cue reexposure in the absence of footshock, i.e. the retest session. Nonetheless, this preclinical work lends support to the proposed anxiolytic/antidepressant potential of this drug class. Future clinical studies with the various tachykinin NK_1 receptor antagonists currently in development will determine whether this potential is to be realised.

Acknowledgements

We gratefully acknowledge the contributions of Drs. Thierry Godel and Heinz Stadler for the synthesis of MK-869, GR73632, CP-99,994 and CP-100,263, and Eva Breitmaier for her assistance in some experiments. We are also indebted to Dr. Andrew Grottick for programming the MED-PC system and for his help in some of the preliminary experiments. We would also like to extend our thanks to Dr. Andrew Sleight for useful discussions regarding this work.

References

Ballard, T.M., Breitmaier, E., Grottick, A.J., Koch, S., Higgins, G.A., 1999. Inhibition of footshock-induced foot tapping behaviour in the gerbil by an NK1 receptor antagonist. J. Psychopharmacol. 13 (3S), 93.

Bannon, M.J., Deutch, A.Y., Tam, S-Y., Zamir, N., Eskay, R.L., Lee, J.M., Maggio, J.E., Roth, R.H., 1986. Mild footshock stress dissociates substance P from substance K and dynorphin from Met- and Leu-enkephalin. Brain Res. 381, 393–396.

Beresford, I.J.M., Birch, P.J., Hagan, R.M., Ireland, S.J., 1991. Investigation into species variants in tachykinin NK_1 receptors by use of the non-peptide antagonist, CP-96,345. Br. J. Pharmacol. 104, 292–293.

Bodnoff, S.R., Suranyi-Cadotte, B., Quirion, R., Meaney, M.J., 1989. A comparison of the effects of diazepam versus several typical and atypical anti-depressant drugs in an animal model of anxiety. Psychopharmacology 97, 277–279.

Bristow, L.J., Young, L., 1994. Chromodacryorrhea and repetitive hind paw tapping: models of peripheral and central tachykinin NK_1 receptor activation in gerbils. Eur. J. Pharmacol. 253, 245–252.

Brodin, E., Rosen, A., Schott, E., Brodin, K., 1994. Effects of sequential removal of rats from a group cage, and of individual housing of rats, on substance P, choleocystokinin and somatostatin levels in the periaqueductal grey and limbic regions. Neuropeptides 26, 253–260.

Clark, M.M., Galef Jr., B.G., 1979. A sensitive period for the maintenance of emotionality in Mongolian gerbils. J. Comp. Physiol. Psychol. 93, 200–210.

Davis, M., 1999. Functional neuroanatomy of anxiety and fear: a focus on the amygdala. In: Charney, D.S., Nestler, E.J., Bunney, B.S. (Eds.), Neurobiology of Mental Illness. Oxford Univ. Press, UK, pp. 463–474.

De Felipe, C., Herrero, J.F., O'Brien, J.A., Palmer, J.A., Doyle, C.A., Smith, A.J.H., Laird, J.M.A., Belmonte, C., Cervero, F., Hunt, S.P., 1998. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature 392, 294–397.

File, S.E., 1997. Anxiolytic action of a neurokinin 1 receptor antagonist in the social interaction test. Phrmacol. Biochem. Behavior 58, 747–752.

Gitter, B.D., Waters, D.C., Bruns, R.F., Mason, N.R., Nixon, J.A., Howbert, J.J., 1991. Species differences in affinities of non-peptide antagonists for substance P receptors. Eur. J. Pharmacol. 197, 237–238.

Graham, E.A., Turpin, M.P., Stubbs, C.M., 1993. Characterisation of the tachykinin-induced hindlimb thumping response in gerbils. Neuropeptides 4, 228.

Griebel, G., Moreau, J.L., Jenck, F., Misslin, R., Martin, J.R., 1994. Acute and chronic treatment with 5-HT reuptake inhibitors differentially modulate emotional responses in anxiety models in rodents. Psychopharmacology 113, 463–470.

Griebel, G., Blanchard, D.C., Agnes, R.S., Blanchard, R.J., 1995. Differential modulation of antipredator defensive behaviour in Swiss–Webster mice following acute or chronic administration of imipramine or fluoxetine. Psychopharmacology 120, 57–66.

Hahn, M.K., Bannon, M.J., 1999. Stress-induced c-*fos* expression in the rat locus coeruleus is dependant on neurokinin 1 receptor activation. Neuroscience 94, 1183–1188.

Hale, J.J., Mills, S.G., MacCoss, M., Finke, P.E., Cascieri, M.A., Sadowski, S., Ber, E., Chicchi, G.G., Kurtz, M., Metzger, J., Eiermann, G., Tsou, N.N., Tattersall, F.D., Rupniak, N.M.J., Williams, A.R., Rycroft, W., Hargreaves, R., MacIntyre, D.E., 1998. Structural optimization affording 2-(*R*)-(1-(*R*)-3,5-Bis(trifluoromethyl)phenylethoxy)-3-(*S*)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine, a potent, orally active, long-acting morpholine acetal human NK-1 receptor antagonist. J. Med. Chem. 41, 4607–4614.

Hendrie, C.A., Starkey, N.J., 1998. Pair-bond disruption in Mongolian gerbils: effects on subsequent social behaviour. Physiol. Behav. 63, 895–901.

Ikeda, K., Miyata, K., Orita, A., Kubota, H., Yamada, T., Tomioka, K., 1995. RP67580, a neurokinin$_1$ receptor antagonist, decreased restraint stress-induced defecation in rat. Neurosci. Lett. 198, 103–106.

Kramer, M.S., Cutler, N., Feighner, J., Shrivastava, R., Carman, J., Sramek, J.J., Reines, S.A., Liu, G., Snavely, D., Wyatt-Knowles, E., Hale, J.J., Mills, S.G., MacCoss, M., Swain, C.J., Harrison, T., Hill, R.G., Hefti, F., Scolnick, E.M., Cascieri, M.A., Chicchi, G.G., Sadowski, S., Williams, A.R., Hewson, L., Smith, D., Carlson, E.J., Hargreaves, R.J., Rupniak, N.M., 1998. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 281, 1640–1645.

LeDoux, J.E., 1998. Fear and the brain: where have we been, and where are we going? Biol. Psychiatry 44, 1229–1238.

LeDoux, J.E., Iwata, J., Cicchetti, P., Reis, D.J., 1988. Different projections of the central amygdaloid nucleus mediate autonomic and behavioural correlates of conditioned fear. J. Neurosci. 8, 2517–2529.

Maren, S., 1999. Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. TINS 22, 561–567.

McLean, S., Ganong, A., Seymour, P.A., Snider, R.M., Desai, M.C., Rosen, T., Bryce, D.K., Longo, K.P., Reynolds, L.S., Robinson, G., Schmidt, A.W., Siok, C., Heym, J., 1993. Pharmacology of CP-99,994; a nonpeptide antagonist of the tachykinin neurokinin-1 receptor. J. Pharmacol. Exp. Ther. 267, 472–479.

Murtra, P., Sheasby, A.M., Hunt, S.P., De Felipe, C., 2000. Rewarding effects of opiates are absent in mice lacking the receptor for substance P. Nature 405, 180–183.

Nutt, D., 1998. Substance-P antagonists: a new treatment for depression? Lancet 352, 1644–1646.

Phillips, R.G., LeDoux, J.M., 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav. Neurosci. 106, 274–285.

Routtenberg, A., Kramis, R.C., 1967. 'Foot-stomping' in the gerbil: rewarding brain stimulation, sexual behaviour, and foot shock. Nature 214, 173–174.

Rupniak, N.M.J., Williams, A.R., 1994. Differential inhibition of foot tapping and chromodacryorrhoea in gerbils by CNS penetrant and non-penetrant tachykinin NK$_1$ receptor antagonists. Eur. J. Pharmacol. 265, 179–183.

Rupniak, N.M.J., Tattersall, F.D., Williams, A.R., Rycroft, W., Carlson, E.J., Cascieri, M.A., Sadowski, S., Ber, E., Hale, J.J., Mills, S.G., MacCoss, M., Seward, E., Huscroft, I., Owen, S., Swain, C.J., Hill, R.G., Hargreaves, R.J., 1997. In vitro and in vivo predictors of the anti-emetic activity of tachykinin NK$_1$ receptor antagonists. Eur. J. Pharmacol. 326, 201–209.

Rupniak, N.M.J., Carlson, E.C., Harrison, T., Oates, B., Seward, E., Owen, S., De Felipe, C., Hunt, S., Wheeldon, A., 2000. Pharmacological blockade or genetic deletion of substance P (NK1) receptors attenuates neonatal vocalisation in guinea-pigs and mice. Neuropharmacology 39, 1413–1421.

Seguin, L., Le Marouille-Girardon, S., Millan, M.J., 1995. Antinociceptive profiles of non-peptidergic neurokinin1 and neurokinin2 receptor antagonists: a comparison to other classes of antinociceptive agent. Pain 61, 325–343.

Shaik, M.B., Steinberg, A., Siegel, A., 1993. Evidence that substance P is utilized in medial amygdaloid facilitation of defensive rage behaviour in the cat. Brain Res. 625, 283–294.

Smith, D.W., Hewson, L., Fuller, P., Williams, A.R., Wheeldon, A., Rupniak, N.M.J., 1999. The substance P antagonist L-760,735 inhibits stress-induced NK1 receptor internalisation in the basolateral amygdala. Brain Res. 848, 90–95.

Tattersall, F.D., Rycroft, W., Hargreaves, R., Hill, R.G., 1993. The tachykinin NK1 receptor antagonist CP-99,994 attenuates cisplatin induced emesis in the ferret. Eur. J. Pharmacol. 250, R5–R6.

Texeira, R.M., Santos, A.R.S., Ribeiro, S.J., Calixto, J.B., Rae, G.A., De Lima, T.C.M., 1996. Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze behavior in mice. Eur. J Pharmacol. 311, 7–14.

Van Praag, H.M., 1988. Serotonin disturbances in psychiatric disorders: functional versus nosological interpretation. Adv. Biol. Psychiatry 17, 52–57.

Westenberg, H.G.M., Den Boer, J.A., 1988. Clinical and biochemical effects of selective serotonin-uptake inhibitors in anxiety disorders. Adv. Biol. Psychiatry 17, 84–99.

Zernig, G., Dietrich, H., Maggi, C.A., Saria, A., 1992. The substance P (NK1) receptor antagonist (±)-CP-96,345 causes sedation and motor impairment in Swiss albino mice in the black-and-white box behavioral paradigm. Neurosci. Lett. 143, 169–172.

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Review

Substance P antagonists: novel agents in the treatment of depression

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The field of neuropeptides has been expanding very rapidly in recent years. Apart from understanding their physiology and elucidating their functional role as putative neurotransmitters, research has focused on producing drugs that may treat a variety of illnesses in a novel way. Substance P antagonists occupy a central role in this area of intensive scientific activity. Substance P (SP), an undecapeptide, is abundant both in the periphery and in the CNS, where it is usually co-localised with one of the classical neurotransmitters, most commonly serotonin (5-HT). A role for SP is proposed in the regulation of pain, asthma, psoriasis, inflammatory bowel disease and, in the CNS, emesis, migraine, schizophrenia, depression and anxiety. A recently published positive study of MK 869, in depression, a novel SP antagonist has generated excitement amongst psychopharmacologists. It is the first time that a drug, not directly related to monoamine transmitters, has showed efficacy in depression. Although MK 869 has been suspended from further development, a host of other compounds, with similar action and better pharmacological profile, are currently under development. In this review, the pharmacology of central SP and its receptors are discussed, together with the exploration of the prospects and implications for future treatments of depression.

Keywords: *depression, MK 869, NK_1 antagonists, substance P*

Exp. Opin. Invest. Drugs (2000) 9(8):1871-1875

1. Introduction

The field of neuropeptides has been progressing rapidly in recent years. New techniques, such as *in situ* hybridisation and antisense probes, have facilitated the study of known neuropeptides and the discovery of new ones. A number of neuropeptide receptors have been fully or partially characterised, while the development of specific receptor ligands (agonists and antagonists) helps to elucidate their functional role. Most research so far has been conducted in animals, but human data, including the testing of experimental drugs for specific indications, have started to accumulate. Despite the progress in knowledge and the development of exciting hypotheses, no major breakthrough in the neuropeptide clinical psychopharmacology has taken place. However, as of last year, the picture is changing. Very promising results from studies assessing the antidepressant potential of substance P (SP) antagonists have been published [1].

SP, an undecapeptide (**Table 1**), is widely distributed in the CNS and it appears to be virtually always co-localised with at least one of the classic neurotransmitters, usually serotonin (5-HT). This led to some interesting hypotheses about its role in these nerve cells. Neurones, affected by the

 ISSN 1354-3784

Table 1: Substance P and mammalian tachykinins.

Tachykinin	Sequence
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2

information they receive, may release a cocktail instead of a single transmitter, depending on differential patterns of afferent firing. In turn, this allows for a broad spectrum of potential actions and differential temporal signalling, faster or slower. In general, neuropeptides, in particular SP, are produced in the ribosomes. Once released, they are replaced by new synthesis, with little or no re-uptake at synaptic level. Their release follows a small elevation in the Ca^{2+} concentration in the cytoplasm, while biogenic amine transmitters are released after higher elevations of Ca^{2+} in the synapses [2].

Depression is one of the areas of psychopathology in which an important functional role for SP is proposed and research for potential antidepressant compounds has been under way for some years. Ideally, in order to prove useful in clinical practice, the SP receptor ligands should be potent and able to cross the blood-brain barrier, have good oral bioavailability and a reasonably long duration of action. This review will focus on the human research carried out using SP antagonists in depressive illness, as well as theoretical prospects and avenues for future research. Full review of the animal literature in this area is beyond the scope of this paper. However, some basic findings will be presented and the reader will be directed to relevant sources covering this topic.

2. Substance P

2.1 Distribution in the CNS

Von Euler and Gaddum discovered SP in 1931. It is the most abundant of the neurokinin (tachykinin) group of peptides, which are defined by the common C-terminal amino acid sequence Phe-X-Gly-Leu-Met-NH_2 (**Table 1**). This group also includes neurokinin A and neurokinin B [3,4]. SP is a major transmitter of small, unmyelinated, primary afferent nerves of the substantia gelatinosa of the spinal cord and the spinal tract of the trigeminal nerve, where it exerts a primary role in pain transmission. Stimulation of SP fibres produces burning pain. In the CNS, SP neurones are present in the tegmental nuclei of the medulla, the central nucleus of amygdala and, notably, in the spiny neurones of the striatum that project to the medial segment of the globus pallidus and the substantia nigra pars reticulata. Fewer SP neurones are present in the dentate gyrus of hippocampus. Some neurones are also present in layers 5 and 6 of the cortex, where they seem to project to the upper layers [5].

2.2 Substance P receptors

The three recognised neurokinin receptors are coupled with G-proteins and they are named NK_1, NK_2 and NK_3, respectively. Of these, NK_1 is the preferred receptor for SP, while neurokinin A has the highest affinity for NK_2 and neurokinin B for NK_3 receptors. Despite earlier thoughts about cross linking between the various tachykinins and their receptors, it has now become obvious that these transmitters are very selective. Therefore, SP should be considered as the only natural ligand of NK_1 receptor [4].

A variety of methods have been used to study the distribution of NK_1 in the brain, including autoradiography, immunohistochemistry and mRNA encoding of the receptor. This receptor is widely distributed both in the periphery, mainly the dorsal horn of the spinal cord and the CNS. NK_1 receptors are widespread in the striatum, the nucleus accumbens, the hypothalamus, the amygdala, the hippocampus, the periaqueductal grey matter, the nucleus of the solitary tract and the raphe nuclei [4-6]. Similar to other neuropeptides, there is considerable divergence between the location of these peptides and their receptors [5]. Thus, areas rich in SP immunoreactive nerve endings have an apparent lack of NK_1 receptors, an example being the substantia nigra. The opposite also occurs. Areas rich in NK_1 receptors, such as the dentate gyrus, are not apparently innervated by SP-containing neurones [4]. The reason for this discrepancy is not yet clear, although it may be due to lack of appropriate technology that could identify 'missing' neurones or their receptors.

2.3 Early development of substance P antagonists and possible indications

NK_1 and SP antagonists have been investigated unsuccessfully for many years, as potential agents in pain relief. Poor bioavailability, low potency, low selectivity and neurotoxicity were common problems with early antagonists resembling SP itself. Early

Table 2: Chemical structure classes of non-peptide substance P antagonists.

Steroids
Perhydroisoindolones
Benzylamino & benzylether quinuclidine
Benzylamino piperidines
Benzylether piperidines
Other piperidine-based structures
Tryptophan-based antagonists

attempts for non-peptide antagonists were hampered by the species specificity of the primary sequence of the NK_1 receptor, which influenced the potency of the studied compounds [4]. This species difference is particularly important with respect to the rat and human receptors. Therefore, appropriate models had to be developed for species other than rats, such as gerbils and guinea-pigs, with NK_1 receptors closer to the human sequence. The development of smaller molecule antagonists in recent years, gave new impetus in this area of research. It has been suggested that, apart from pain, the NK_1 antagonists may have a role to play in a number of conditions. This applies both to the periphery (inflammatory bowel disease, cystitis, psoriasis, asthma), and the CNS (emesis, migraine, schizophrenia, movement disorders, Alzheimer's, Parkinson's, multiple sclerosis, depression, and anxiety) [3,7-9].

3. Chemical classification of non-peptide substance P antagonists

Since the early 1990s, when the first SP non-peptide antagonists were reported [10,11], the field has seen a proliferation of new compounds [7,12-15]. These drugs belong to a number of different classes: steroids, perhydroisoindolones, benzylamino and benzylether quinuclidine, benzylamino piperidines, benzylether piperidines, other piperidine-based structures and tryptophan based antagonists (**Table 2**). These classes have been extensively described by Quartara and Maggi [12]. The reader is referred to this excellent review for a thorough description of the evolution of each subgroup and their respective pharmacological properties. We were able to identify only two compounds that progressed to Phase II trials in depression (**Table 3**).

Table 3: NK_1 antagonists in development for depression.

Chemical name	Phase	Country	Originator
MK 869	II (suspended)	USA	Merck
NKP 608	II	USA	Novartis

3.1 MK 869

MK 869, also known as L 754,030, was the first SP antagonist to enter clinical trials for depression. The chemical structure of the compound is {5-(2(*R*)-(1*R*)-(3,5-*Bis*(trifluoromethyl)phenyl)ethoxy) -3(*S*)-(4-fluorophenyl) morpholin-4-ylmethyl) 3,4-dihydro-2H-1,2,4-triazol-3-one [7]. This agent is specific for the human NK_1 receptor, showing no affinity for monoamine oxidases, noradrenaline, serotonin or dopamine re-uptake sites, or receptors and transporters of opiates [1]. Despite early positive results (see section 4.3), the development of this drug for depression has been suspended, while Phase II clinical trials for anxiety disorders and schizophrenia continue. This compound has also been tested for migraine and emesis [16].

3.2 NKP 608

Little is in the public domain about this compound, other that it is currently in Phase II trials for depression and social phobia, as well as chronic bronchitis.

4. Substance P antagonists in depression

4.1 Preclinical evidence and theoretical concepts

SP is released in the CNS after noxious stimulation. Following this finding, it was hypothesised that inherent hyperactivity of SP release may also account for the 'emotional pain' described by depressed and anxious patients. The presence of SP in areas associated with anxiety, such as the amygdala, the hippocampus, the hypothalamus, and the periaqueductal grey matter, as well as the co-localisation with serotonin in raphe nuclei (see section 4.3), strengthened the view of involvement of this neuropeptide in affective disorders. Activation of central SP pathways, by means of injecting SP agonists, generates an array of behavioural changes in animals, similar to the ones seen in anxiety and depression animal models. There is also evidence that antidepressants and anxiolytics cause downregulation of SP. There is very little evidence in humans regarding the above. In one study, CSF levels of SP were found increased in

depressed patients, but the results were equivocal and they have not been replicated [7,17].

4.2 Evidence from experimental drugs in humans

The limited human data of SP receptor ligands created excitement when they were published [1,3,18], since the results potentially signified a major breakthrough in the human neuropeptide psychopharmacology and the development of the first antidepressant drug not related to monoamine function. MK 869 (see section 3.1) was tested in the treatment of moderate to severe depression, in a proof-of-concept study. In four sites, the experimental drug was used at a single dose of 300 mg daily and its effect was comparable with that of a moderate clinical dose (20 mg/day) of paroxetine, a selective serotonin re-uptake inhibitor and significantly better than placebo. The drug was safe and well-tolerated, with notable absence of sexual dysfunction in the MK 869 group [1,7]. Fewer patients on MK 869 or placebo discontinued due to side effects (3 and 4% respectively) compared with paroxetine (19%). This antidepressant effect appeared to be independent of any augmentation of serotonin or noradrenaline function, but the fact that the efficacy of MK 869, as in other antidepressants, was expressed two to three weeks following the onset of treatment, suggested the possibility of a final common pathway for the action of antidepressants [3,18]. What was also of great interest was that MK 869 appeared to treat co-morbid anxiety very effectively [1,18].

Unfortunately, subsequent studies were plagued by high placebo response and a decision was taken to suspend the development of the drug for the indication of depression and continue with studies in anxiety [19]. Poor oral bioavailability also meant that higher doses would be needed, to the detriment of the side effect profile. A follow-up compound, which is said to be more potent, is in the pipeline.

4.3 Mechanism of action of substance P antagonists in depression

It appears that the putative antidepressant action of SP antagonists in depression is exerted through interaction with the 5-HT systems of the brain. This is supported mainly from animal studies, but important human data are gradually emerging. Serotonin interacts extensively with SP. As mentioned earlier, studies have shown that serotonin and SP are co-localised in a substantial part of the raphe neurones in the human brain [20]. Almost 50% of the serotonin neurones in the dorsal raphe, which send widespread projections to the forebrain and 25% of 5-HT neurones in the median raphe nucleus, express SP mRNA [21].

However, it is possible that the antidepressant effect of SP antagonists may be due to other mechanisms, such as interaction with the serotonin systems in areas other than the raphe nuclei. Local SP neurones in limbic areas may play an important role in this action [21]. Further, other neurotransmitter systems may be involved. There is animal evidence that SP may be involved in the activation of the noradrenergic system of locus coeruleus, following stress. Intracerebral infusion of SP antagonists inhibited the activation of the locus coeruleus caused by immobilisation in rats [7]. The relationship of dopamine and depression has long been the focus of attention [22]. In the striatum, animal data suggest that, although the principal input of the SP/dynorphin neurones projecting to substantia nigra is GABA, these neurones are also under regulation by dopamine [2]. What this illustrates is, simply, the complexity of the regulation of the chemical neurotransmission that is thought to play a key role in depression.

5. Expert opinion

Depression is one of the major health problems in the world [23]. Despite their undoubted effectiveness, none of the current treatments shows efficacy of more than 70%. Therefore, there is a need for alternative pharmacological approaches that will help to improve this outcome. The link of SP and depression was rather serendipitous to begin with, like most of the important discoveries in psychopharmacology. The study of SP antagonists in depression has been a very active area of research in recent years. Although little has been achieved so far in terms of producing definite effective treatments, there have been considerable advances in our knowledge and exciting new agents are under development. Some of the problems of previous compounds, such as poor bioavailability, are now probably solved, and the future looks promising [18].

The way forward probably stems from a thorough understanding of the relationship between SP and classical neurotransmitters in general and in specific areas of the brain implicated in the psychopathology of depression. Perhaps the most important contribution of the neuropeptide study, so far, has been to

dispel the simplistic idea that one single transmitter may be responsible for as wide a phenomenon as depression. The idea that each transmitter can modify the response of neurones to its co-transmitters is gaining popularity [24], but it opens a seemingly infinite repertoire of combinations, which may look bewildering. The clarification of these complex inter-relationships, though, may shed some light to the pathophysiology of depression and lead to more effectively designed treatments.

Bibliography

Papers of special note have been highlighted as:
- • of interest

1. KRAMER MS, CUTLER NR, FEIGHNER J *et al.*: **Distinct mechanism for antidepressant activity by blockade of central substance P receptors.** *Science* (1998) **281**:1640-1645.
   - • The first and only positive study of a SP antagonist in depression.
2. HOKFELT TGM, CASTEL M-N, MORINO P, ZHANG X, DAGERLIND A: **General overview of neuropeptides.** In: *Psychopharmacology. The fourth generation of progress.* Bloom FE, Kupfer DJ (Eds.), Raven Press, New York, USA (1995):483-492.
   - • Useful general overview of the neuropeptide field.
3. WAHLESTEDT C: **Reward for persistence in substance P research.** *Science* (1998) **281**:1624-1625.
4. SARIA A: **The tachykinin NK_1 receptor in the brain: pharmacology and putative functions.** *Eur. J. Pharmacol.* (1999) **375**: 51-60.
   - • Useful review of the NK_1 receptor.
5. PENNEY JB JR.: **Neurochemical neuroanatomy.** In: *Neuropsychiatry.* Fogel BS, Schiffer RB, Rao SM (Eds.), Williams & Wilkins, Baltimore, USA (1996):145-171.
6. OTSUKA M, YOSHIOKA K: **Neurotransmitter functions of mammalian tachykinins.** *Physiol. Rev.* (1993) **73**:229-308.
7. RUPNIAK NM, KRAMER MS: **Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK_1) antagonists.** *Trends Pharmacol. Sci.* (1999) **20**:485-490.
   - • Conceptual review of developments in SP research from two of the pioneers in the field.
8. BARKER R: **Substance P and neurodegenerative disorders: a speculative review.** *Neuropeptides* (1991) **20**:73-78.
9. BARKER R, LARNER A: **Substance P and multiple sclerosis.** *Med. Hypotheses* (1992) **37**:40-43.
10. MAGGI CA, PATACCHINI R, ROVERO P, GIACHETTI A: **Tachykinin receptors and receptor antagonists.** *J. Auton. Pharmacol.* (1993) **13**:958-962.
11. REGOLI D, BOUDON A, FAUCHERE J: **Receptors and antagonists for substance P and related peptides.** *Pharmacol. Rev.* (1994) **46**:551-599.
12. QUARTARA L, MAGGI CA: **The tachykinin NK_1 receptor. Part I: ligands and mechanisms of cellular activation.** *Neuropeptides* (1997) **31**:537-563.
    - • Thorough review of NK_1 ligands.
13. IYENGAR S, HIPSKIND PA, GEHLERT DR *et al.*: **LY303870, a centrally active neurokinin-1 antagonist with a long duration of action.** *J. Pharmacol. Exp. Ther.* (1997) **280**:774-785.
14. HOSOKI R, YANAGISAWA M, ONISHI Y, YOSHIOKA K, OTSUKA M: **Pharmacological profiles of new orally active nonpeptide tachykinin NK_1 receptor antagonists.** *Eur. J. Pharmacol.* (1998) **341**:235-241.
15. WALPOLE CSJ, BROWN MCS, JAMES IF *et al.*: **Comparative, general pharmacology of SDZ NKT 343, a novel, selective NK_1 receptor antagonist.** *Br. J. Pharmacol.* (1998) **124**:83-92.
16. NAVARI RM, REINHARDT RR, GRALLA RJ *et al.*: **Reduction of cisplastin-induced emesis by a selective neurokinin-1-receptor antagonist.** *N. Engl. J. Med.* (1999) **340**:190-195.
17. BERRETTINI WH, RUBINOW PR, NURNBERGER JI, SIMMONS-ALLING S, POST RM, GERSHON ES: **CSF substance P immunoreactivity in affective disorders.** *Biol. Psychiat.* (1985) **20**:965-968.
18. NUTT DJ: **Substance P antagonists: a new treatment for depression?** *Lancet* (1998) **352**:1644-1646.
    - • Discussion of the future directions in SP filed in depression and anxiety.
19. EDITORIAL: **Delay for Merck's substance P blocker in depression.** *SCRIPT* (1999) **2406**:20.
20. BAKER KG, HALLIDAY GM, HORNUNG J-P, GEFFEN LB, COTTON RG, TORK I: **Distribution, morphology and number of monoamine-synthetising and substance P containing neurons in the human dorsal raphe nucleus.** *Neuroscience* (1991) **42**:757-775.
21. SERGEYEV V, HOKFELT T, HURD Y: **Serotonin and substance P co-exist in dorsal raphe neurons of the human brain.** *NeuroRep.* (1999) **10**: 3967-3970.
    - • Review and important new evidence for the co-localisation of SP and 5-HT.
22. ARGYROPOULOS SV, NUTT DJ: **Anhedonia and chronic mild stress model of depression.** *Psychopharmacol.* (1997) **134**:333-336.
23. MURRAY CJL, LOPEZ AD: **Alternative projections of mortality and disability by cause 1990-2020: global burden of disease study.** *Lancet* (1997) **349**:1498-1504.
24. FULLER RW: **Mechanisms and functions of serotonin neuronal systems.** *Ann. NY Acad. Sci.* (1996) **780**:176-184.

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CNS Drugs 2005; 19 (4): 275-293
1172-7047/05/0004-0275/$34.95/0

Substance P Receptor Antagonists in Psychiatry

Rationale for Development and Therapeutic Potential

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Abstract

Increasing evidence suggests that substance P (SP) and its receptor (neurokinin [NK]-1 receptor [NK1R]) might play an important role in the modulation of stress-related, affective and/or anxious behaviour. First, SP and NK1R are expressed in brain regions that are involved in stress, fear and affective response (e.g. amygdala, hippocampus, hypothalamus and frontal cortex). Second, the SP content in these areas changes upon application of stressful stimuli. Third, the central administration of SP produces a range of fear-related behaviours. In addition, the SP/NK1R system shows significant spatial overlap with neurotransmitters such as serotonin and noradrenaline (norepinephrine), which are known to be involved in the regulation of stress, mood and anxiety. Therefore, it was hypothesised that blockade of the NK1R might have anxiolytic as well as antidepressant effects.

Preclinical studies investigating the effects of genetic or pharmacological NK1R inactivation on animal behaviour in assays relevant to depression and anxiety revealed that the behavioural changes resemble those seen with reference antidepressant or anxiolytic drugs. Furthermore, antagonism or genetic inactivation of the NK1R causes alterations in serotonin and norepinephrine neuronal transmission that are likely to contribute to the antidepressant/anxiolytic activity of NK1R antagonists but that are – at least partially – distinct from those produced by established antidepressant drugs. This underlines the conceivable unique mechanism of action of this new class of compounds. In three independent clinical trials with three different compounds (aprepitant [MK-869], L-759274 and CP-122721), an antidepressant effect of NK1R antagonists could be demonstrated. These results, however, have been challenged by recent failed studies with aprepitant.

There are numerous indications from preclinical studies that, in addition to SP and NK1R, other neurokinins and/or neurokinin receptors might also be involved in the modulation of stress-related behaviour and that exclusive blockade of the NK1R might not be sufficient to produce consistent anxiolytic and antidepressant effects. One such candidate is the neurokinin-2 receptor (NK2R), and clinical trials to assess the antidepressant effects of NK2R antagonists are currently underway. Of special interest might also be substances that block more than one receptor type such as NK1/2R antagonists or NK1/2/3R antagonists. These

compounds may be more efficacious in antagonising the effects of SP than compounds that only block the NK1R.

1. Current Challenges in the Treatment of Depression and Anxiety

Depression and anxiety are not only the most common psychiatric disorders, they are also one of the leading causes of disability in general (measured by the number of years lived with a disabling condition), worldwide.[1] Drugs that are currently available for the pharmacotherapy of depression include SSRIs, serotonin and noradrenaline (norepinephrine) reuptake inhibitors (SNRIs), tri- and tetracyclic antidepressants (TCAs) and MAOIs. Principally, they all act via noradrenergic or serotonergic neurotransmitter systems in the CNS. Although clearly effective, all of these drugs show substantial drawbacks: it can take up to 3–4 weeks to produce a significant improvement in symptoms; 30% of patients do not respond at all; an even higher percentage show only partial response; and a variety of adverse effects lead to non-compliance. With regard to the pharmacotherapy of anxiety disorders, benzodiazepines, with their fast onset of action and high efficacy, may be suitable for acute treatment. Their addictive potential and other adverse effects such as sedation and cognitive impairment, however, limit their suitability as long-term medication. For the long-term pharmacotherapy of anxiety disorders, antidepressants are currently used most commonly but, again, not without the above-mentioned drawbacks.

In order to achieve not only higher response and remission rates, but also a more reliable relapse prevention, there still exists the need for drugs (or drug combinations) with improved short-term efficacy plus a better long-term tolerability, both of which ensure more compliance. These are three important requirements for successful pharmacotherapy of chronic conditions such as depression and anxiety disorders. Therefore, active investigations continue in the search for antidepressant and/or anxiolytic agents with novel mechanisms of action. Currently, great hopes are placed on neuropeptidergic systems, one of them being the extensively studied substance P (SP)/neurokinin (NK)-1 receptor (NK1R) pathway.[2]

2. Overview of Pharmacology of Substance P (SP) and the Neurokinin-1 Receptor (NK1R)

The neuropeptide SP (discovered by Von Euler and Gaddum[3] in 1931) consists of 11 amino acids.[4] Together with neurokinin A (NKA), neurokinin B (NKB) and the recently identified haemokinin-1 (HK-1),[5,6] SP belongs to the family of mammalian neurokinin (formerly tachykinin) peptides that are defined by their common carboxy-terminal sequence Phe-X-Gly-Leu-MetNH2 (table I). SP can be synthesised from four alternatively spliced forms of the tachykinin precursor 1 (*TAC1*) gene,[7-9] with the β- and γ-splice variants also containing the coding sequence for NKA. NKB is formed from a separate gene, tachykinin 3 (*TAC3*),[10] and HK-1 is formed from tachykinin 4 (*TAC4*)[11] (in the mouse, the gene coding for NKB is *TAC2*). In contrast to classic neurotransmitters, which are synthesised locally in the nerve terminals and which, upon release, can be recycled easily by specific membrane reuptake mechanisms, peptides can only be restored via time-consuming ribosomal

Table I. Amino acid sequence of substance P (SP), mouse/rat haemokinin-1 (m/rHK-1), human haemokinin-1 (hHK-1), neurokinin A (NKA) and neurokinin B (NKB)

Neurokinin	Sequence	Preferred receptor
SP	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH2	NK1R
hHK-1	Thr-Gly-Lys-Ala-Ser-Gln-Phe-Phe-Gly-Leu-MetNH2	NK1R
m/rHK-1	Arg-Ser-Arg-Thr-Arg-Gln-Phe-Tyr-Gly-Leu-MetNH2	NK1R
NKA	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-MetNH2	NK2R
NKB	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-MetNH2	NK3R

NK1R = neurokinin-1 receptor; **NK2R** = neurokinin-2 receptor; **NK3R** = neurokinin-3 receptor.

de novo synthesis in the cell body and dendrites and subsequent transport to their release sites.[12]

SP, which is stored in 'large dense core' vesicles and which was shown to localise to synaptic vesicles,[13,14] is released by Ca^{2+}-dependent exocytosis. Since the release of peptides only requires small elevations in the Ca^{2+} concentration, SP might be released not only into the synaptic cleft, but probably also extrasynaptically.[15,16]

The neurokinins exert their effects by binding to the G-protein-coupled neurokinin receptors. So far, three mammalian neurokinin receptors have been identified, each with a preferred ligand. SP and HK-1 bind preferentially to the NK1R, whereas NKA and NKB show preference for the NK2 and NK3 receptors (NK2R and NK3R), respectively (table I).[6,17] However, each neurokinin possesses agonist properties at all three receptor types. Like all G-protein-coupled receptors, the NK1R contains seven transmembrane helical segments and activates second-messenger systems (e.g. phosphatidyl inositol, arachidonic acid and cyclic adenosine monophosphate [cyclic AMP]) via G-proteins. Species-dependent variations exist in the amino acid sequence of the NK1R protein. Remarkably, these variations do not affect the potency or efficacy of SP. However, they determine the species-related differences in the potency of nonpeptide antagonists, which have different binding sites compared with the agonists.[18]

The NK1R is distributed in the plasma membrane of cell bodies and dendrites of unstimulated neurons, but upon SP binding and signal transduction, the SP/NK1R complex undergoes rapid endosomal internalisation. SP is then degraded, whereas the NK1R recycles back to the cell surface.[19,20] In functional studies, this receptor internalisation can be used as a marker for SP release.[21-23] Several proteolytic enzymes have been found to be capable of rapidly degrading SP, including neutral endopeptidase 24.11, metalloendopeptidase, dipeptidyl-peptidase IV, acetylcholinesterase and angiotensin-converting enzyme.[24,25]

SP-containing cell bodies and nerve fibres are vastly present in the peripheral nervous system (PNS) as well as in the CNS. In the human CNS, SP can be found, for example, in the cerebral cortex, the striatum, the amygdala, the mammillary bodies, the hippocampus, the septum and nucleus accumbens, the hypothalamus, the periaqueductal grey, the substantia nigra, the raphe nuclei, the locus coeruleus, the nucleus tractus solitarii and the spinal cord.[26-29]

Like all neuropeptides, SP is usually co-localised with classic neurotransmitters (and sometimes other neuropeptides) and thus functions as a so-called 'co-transmitter'. That means that, upon neuronal stimulation, SP is released in concert with the co-localised neurotransmitter and may modulate the transmitter's effects.[30] Examples are co-localisation with glutamate in primary sensory neurons,[31,32] with corticotropin-releasing factor in the hypothalamus, with acetylcholine and GABA in the cortex, with dopamine in the midbrain and striatum, and with serotonin in the raphe nucleus.[33,34] To our knowledge, so far, no study has shown a co-localisation of SP with norepinephrine in any part of the nervous system.

NK1Rs are spread abundantly in the CNS, in many cases overlapping with the distribution of SP.[35] In contrast to the receptors for classic neurotransmitters, which are expressed by almost all neurons in a given CNS region, NK1Rs are expressed by only 5–7% of neurons.[36] In the human CNS, high NK1R densities are found particularly in the spinal cord, the striatum, the nucleus accumbens, the amygdalo-hippocampal area and the septum. However, brain regions such as the cerebral cortex, the hypothalamus, the periaqueductal grey, the locus coeruleus, the raphe nuclei and other brainstem structures also contain substantial NK1R densities.[37-39] Investigating the prefrontal and visual cortex with an immunohistochemical assay, Tooney et al.[40] showed that NK1Rs are only located in the superficial laminae (I–III), but not in the deeper cortical layers. In the rat locus coeruleus, the majority of noradrenergic neurons carry NK1Rs,[41] whereas, in the raphe nuclei, NK1Rs are not found on serotonergic neurons, but on GABAergic and glutamatergic neurons.[42,43]

The physiological role of SP has not been fully clarified yet. It seems to be involved in many physiological and pathophysiological mechanisms. The fact that neurokinins can produce a fast, smooth muscle contraction initially gave them the name 'tachykinins' (in contrast to 'bradykinins'). In the PNS, neurokinin receptors are, for example, expressed by intrinsic enteric neurons, extrinsic prima-

ry afferent nerve fibres, smooth muscle cells and cells of the vascular and the immune system.[44-46] Thus, they enable SP to induce vasodilation and plasma protein extravasation in response to noxious or inflammatory stimuli and to influence electrolyte and fluid secretion of the gut and of other glandular systems (e.g. salivary or pancreatic). In addition, SP modulates the motility of the gastrointestinal and the genitourinary tract and stimulates the ventilatory response to hypoxia. In the spinal cord, SP plays a role in nociception and sensitisation 'wind-up' phenomena, in which input signals to the dorsal horn are being amplified and prolonged.[47] Also, spinal NK1Rs modulate autonomic reflexes such as micturition. In the brain – as this article will review in further detail – SP/NK1R has been suggested to play an important role in the control of various behavioural responses such as stress response and affective and anxiety-like behaviour. At the brainstem level, NK1Rs are involved in the regulation of autonomic reflexes such as emesis and cardiovascular and respiratory function (central administration of an NK1R agonist increases blood pressure and heart and respiratory rate).[48]

In addition to these direct transmitter-like functions and modulatory effects on the actions of neurotransmitters, SP also exerts trophic effects such as stimulating the growth of fibroblasts, smooth muscle cells and axons.[49-51].

3. The Role of SP and the NK1R in the Etiopathology of Psychiatric Disorders

On the basis of its anatomical distribution, its neuromodulatory effects, its involvement in neurogenic inflammation[52-54] and results from functional studies, SP has been proposed to be involved in the etiopathology of a wide variety of pathophysiological conditions. Examples include pain syndromes (e.g. arthritis, peripheral neuropathy, migraine and fibromyalgia), asthma, psoriasis, allergic skin reactions, inflammatory bowel disease, cystitis, incontinence and emesis. Moreover, it has been suggested that SP is important in the etiopathology of psychiatric disorders including neurodegenerative disorders,[55] affective disorders, anxiety disorders and schizophrenia.[48,56-58]

This review focuses on the role of SP in the pathogenesis of affective and anxiety disorders.

3.1 Evidence from Preclinical Studies

3.1.1 Effects of Acute or Chronic Stressors on SP and the NK1R

The localisation of the SP/NK1R system in brain regions involved in the regulation of affective behaviours and the neurochemical response to stress, together with the significant spatial overlap with serotonergic and noradrenergic pathways, indicates that the SP/NK1R pathway may be involved in the modulation of affective behaviours. In fact, numerous alterations in SP synthesis/release and NK1R activation can be observed following exposure of laboratory animals to acute or chronic stressors. Table II summarises the effects of various stressful events on SP tissue levels or SP immunoreactivity in distinct brain areas in the rat. All listed CNS regions play an important role in motivational and reward mechanisms as well as in the fear response and, as such, might be of potential relevance to affective and anxiety disorders. At first sight, the changes in SP content seem to be rather inconsistent. However, considering that bursts of synaptic SP release may be followed by acute SP depletion due to internalisation, immediate peptidase activity and delayed ribosomal SP *de novo* synthesis (see section 2), it becomes understandable that the patterns of SP content are very sensitive to the timing and the precise nature of the stressor.

In addition, immobilisation leads to NK1R internalisation in the amygdala of rats[23] and gerbils,[21] which reflects previous SP binding and receptor activation. Comparable results have been found in guinea-pig pups upon maternal separation.[22] Moreover, it has been shown that the degree of SP release and NK1R internalisation is proportional to the intensity and frequency of a noxious or stressful/aversive stimulus, which Allen et al.,[67] for example, demonstrated for thermal stimuli.

In addition, by applying mild electric foot shocks, Vaupel et al.[68] discovered that stressful stimuli cause not only the above-mentioned alterations in central SP levels, but also a marked increase in adrenal SP release.

Although measuring SP tissue content and receptor internalisation seem to be rather crude methods of assessing the activity of neuropeptidergic circuits, the above-described results taken together are sug-

Table II. Effects of acute or chronic stressors on substance P (SP) tissue levels or SP immunoreactivity in different CNS regions in the rat

Stressor	Investigated CNS area	SP content	References
Intermittent foot shock	VTA, olfactory tubercle, hypothalamic nuclei	↓	59-62
	Septum, hippocampus	↑	
Whole body vibration	Frontal cortex	↓	63
	Nucleus accumbens, amygdala	↑	
Immobilisation (1 hour)	Septum, striatum, hippocampus	↓	23
Isolation (1 day or 1 week)	Dorsal periaqueductal grey	(↑)	64
	Hippocampus, amygdala	↔	
Saline injection	Periaqueductal grey	↑	65
	Nucleus accumbens	↓	
Adjuvant-induction of arthritis (14 days)	Median eminence/arcuate nucleus, and paraventricular nucleus of the hypothalamus	↑	66

VTA = ventral tegmental area; (↑) = modest increase, ↑ = increase, ↓ = decrease, ↔ = unchanged.

gestive of an activation of SP-related pathways by stressful events. This conclusion is further supported by a very recent study by Ebner et al.,[69] who used *in vivo* micropush–pull superfusion and microdialysis techniques to assess directly the dynamics of local SP release in rats. They demonstrated, for the first time, emotional stress-induced SP release in the medial amygdala, which seems considerably more pronounced and prolonged after a severe emotional stressor (immobilisation for 20 minutes) than in response to a rather mild stressor (elevated platform exposure).

3.1.2 Behavioural Responses of Laboratory Animals After Administration of SP (or SP-Like Compounds) and After Genetic Deletion of SP

Results of numerous studies suggest that neurokinin receptor activation can exert multiple behavioural effects depending on the specific brain regions involved. In several CNS regions, it may produce aversion and/or anxiety-like states, as summarised in table III.

In some other CNS areas, however, neurokinin receptor activation may lead to rather opposite effects. For example, microinjections of SP into the rat nucleus basalis magnocellularis produces place preference[83,84] and anxiolytic effects in the elevated plus-maze, as well as the social interaction test.[85]

Bilkei-Gorzo et al.[86] studied mice with a targeted deletion of the *TAC1* gene (i.e. mice that do not synthesise any SP or NKA) in behavioural models relevant to depression and anxiety disorders. In all tests (forced swim test, tail suspension test, novelty suppressed feeding, open-field arena, social interaction test, elevated zero-maze), the *TAC1* mutant mice were more active and showed less anxiety-like behaviour when compared with their wildtype counterparts.

In summary, these results strongly support the idea that the SP/NK1R system might play an important role in the etiopathology of affective and anxiety disorders.

3.1.3 Effects of Antidepressants on SP and the NK1R

The application of various established antidepressants for 14 days did not lead to any alterations in SP concentrations in the investigated rat brain areas,[87-90] except for a reduced SP tissue level in the frontal cortex after 14 days of imipramine.[90] In contrast, Shirayama et al.[91] described a reduction in SP tissue levels in several rat brain regions after 40 days of treatment with the respective antidepressants. These discrepancies might be due not only to the prolonged treatment period, but also to differences in doses, routes of drug administration and the SP assays. On the other hand, acute administration of the SSRI alaproclate, for example, led to an increase in tissue concentrations and release of SP and NKA in the periaqueductal grey in rats,[92] whereas acute treatment with diazepam reduced SP concentrations in the rostral hippocampus and dorsal periaqueductal grey.[64] Measuring the expression of *TAC1* messenger RNA upon subchronic administration of the SSRI zimelidine, Walker et al.[93] observed increased expression levels in the neostriatum, and Riley et al.[94] observed decreased levels in medullary raphe neurons.

With regard to the NK1R, Sartori et al.[95] investigated the effects of long-term antidepressant treatment on NK1R expression in the rat brain. They administered four different established antidepressants for 14 and 42 days and measured their effect on NK1R expression as well as on the number of SP binding sites. None of the treatments caused any significant changes except for tranylcypromine, which increased the number of SP binding sites in the locus coeruleus after 42 days of treatment.

3.2 Evidence from Clinical Studies

3.2.1 SP in the CSF of Depressed Patients

There exist no methods for directly measuring the release of neuropeptides, transmitters or metabolites in human brains; instead, indirect measures such as 'content in the CSF or serum' are being applied.

Rimon et al.[96] measured SP in the CSF of 12 depressed and 12 schizophrenic patients as well as 15 controls. They found significantly increased SP-like immunoreactivity in the depressed patients compared with the schizophrenic patients and the controls. In addition, electrophoresis patterns indicated an increase in SP degradation [SP(1-7)-fragments] in the depressed group, which was interpreted to be of possible pathogenic relevance. Another study from the same group[97] with ten schizophrenic patients, ten matched patients with other psychiatric disorders and ten control subjects did not show any significant difference regarding their CSF SP levels. However, it was noted that in the three patients with depression, the mean SP content was clearly higher than the corresponding mean concentration in the patients in the non-schizophrenic group with other psychiatric disorders, although this difference did not reach statistical significance. In both studies, the CSF SP levels in schizophrenic patients were unaltered relative to controls. Berrettini et al.,[98] however, could not replicate the findings of increased CSF SP in depressed patients. They measured SP levels in the CSF of 19 inpatients (three acutely and unmedicated manic, 12 depressed and four euthymic patients) and 29 outpatients (15 unmedicated bipolar patients and 24 lithium-treated bipolar patients) and found no difference compared with levels in control subjects. On the other hand, Toresson et al.[99] reported that SP itself was not present in measurable concentrations in the CSF (<0.1 pmol/L), but that, instead, an N-terminally elongated form of SP could be measured by a combined high-performance liquid chromatography and radioimmunoassay method. In a study of nine un-

Table III. Aversive, stress-inducing effects of the CNS administration of substance P (SP) or SP-like compounds

Species	Injected substance	Locus of injection	Effect	References
Rat	SP-analogue	ICV	Conditioned place aversion	70
Rat	SP	Periaqueductal grey	Anxiogenic effects in EPM and conditioned place aversion	71,72
Rat	SP	Lateral septal nucleus	Aversive, anxiogenic-like effects in EPM	73
Rat	SP	Medial nucleus of the amygdala	Anxiogenic effects in EPM	69
Mouse	SP, NKA, NK1R- or NK2R-selective agonists	ICV	Anxiogenic effects in EPM	74
Rat	SP	Caudal pontine reticular nucleus	Potentiation of acoustic startle response	75
Guinea-pig	NK1R-agonist	ICV	Distress vocalisations	22
Guinea-pig	SP or selective NK1R-agonists	ICV	Locomotor hyperactivity, wet-dog shakes and face-washing	76,77
Gerbil	NK1R-agonists	ICV	Hind paw tapping[a]	78
Rat, mouse	SP	ICV	Grooming, scratching	79,80
Rat	SP	Dorsal raphe nucleus or ICV	Increase of heart rate and blood pressure	81,82

a An anxiety-like stress behaviour that is recognised as an alarm signal in desert rodents.

EPM = elevated plus-maze; **ICV** = intracerebroventricular; **NK1R** = neurokinin-1 receptor; **NK2R** = neurokinin-2 receptor; **NKA** = neurokinin A.

medicated, depressed inpatients,[100] they showed that the mean level of N-terminally extended SP in CSF was unaffected by a 6-week fluoxetine treatment. However, the pretreatment level correlated significantly with the pretreatment CSF level of the norepinephrine metabolite 4-hydroxy-3-methoxyphenylglycol. Since the study unfortunately did not include any control subjects, it is unknown whether the CSF level of N-terminally extended SP is altered in depression.

With fibromyalgia syndrome, a chronic pain disorder with pathophysiological mechanisms that are often related to depression,[101] elevated CSF SP levels could be demonstrated in five independently conducted studies (reviewed in Russell[102]).

3.2.2 Serum Levels of SP in Response to Stress

Schedlowski et al.[103] investigated the anxiety levels and the SP plasma concentrations in 47 inexperienced tandem parachutists 2 hours before, immediately after and 1 hour after a parachute jump. SP levels were not affected by the jump itself. However, subjects with higher anxiety levels immediately before the jump showed higher SP values throughout the study as compared with the other jumpers.

Weiss et al.[104] measured various immunological, neuroendocrine and psychological parameters as well as plasma SP levels in 22 civilians during and after Scud missile attacks on Israeli cities within the 1991 Persian Gulf War. During the war, median SP levels were significantly elevated.

3.2.3 Serum Levels of SP in Depressed Patients and Modulation of SP Levels in the Course of Antidepressant Treatment

In a study with 23 depressed patients and 33 control subjects, Bondy et al.[105] measured serum SP levels before and after 2 and 4 weeks of antidepressant therapy. Before treatment, the patients with depression displayed significantly higher SP levels than the controls. In control subjects, SP remained relatively constant over a period of 4 weeks. In depressed patients also, there was no overall change in the mean SP level. However, 37% of these patients showed an SP decrease that could be correlated to a better drug response. It has been suggested that the latter proportion of patients might represent a subgroup with the disorder, in whom neuropeptides play a key role, and thus may be candidates for treatment with neurokinin receptor antagonists.

These findings are in line with data from a study in which serum SP levels were determined before and during a 9-week treatment period with paroxetine in combination with either lamotrigine (n = 20) or placebo (n = 20) in 40 depressed patients.[106] In the total group of patients, the mean SP levels did not change during the 9-week trial. However, therapy responders and non-responders differed significantly in their SP serum levels. Responders started with higher SP levels that decreased during drug therapy, whereas non-responders had lower SP levels that increased at the beginning of drug therapy. This may indicate that alterations in SP serum levels are related to drug effects and treatment outcome.

To replicate the above-mentioned findings and to extend longitudinal SP measurements in a larger group of depressed patients, we performed a second study.[107] In 78 depressed patients, serum SP levels were measured before and after 2 and 5 weeks of antidepressant therapy. In addition, CSF SP levels were determined in 11 patients before and after treatment as well as in 11 healthy control subjects. CSF SP levels did not differ between controls and depressed patients, and, as in the above-mentioned studies,[100,105,106] CSF and serum SP levels did not change during the 5 weeks of pharmacotherapy in the total group of patients. In contrast to the study by Bondy et al.[105] and our previous study, however, this most recent study could not confirm the differences between the serum SP levels of responders and non-responders.

These inconsistent clinical findings mirror the results from the above-mentioned preclinical studies, which showed a rather complex and time-sensitive interrelation of antidepressant drug treatment and changes in SP levels (see section 3.1.3). In summary, it is still hard to draw a final conclusion regarding the relevance of SP/NK1R pathways within the therapeutic action of established antidepressants.

3.2.4 Intravenous Administration of SP in Humans

In animal studies, the central administration of SP may produce aversion and/or anxiety-like states

(see section 3.1.2). In human studies, central application is not an option. However, Freed et al.[108] demonstrated that SP is capable of crossing the blood-brain barrier in both directions through an active, energy-dependent transport mechanism. In addition, Clark et al.,[109] measuring – in parallel – SP levels in the plasma and in the CSF of 37 patients who underwent lumbar puncture for various diagnostic purposes, found a close correlation between the plasma and the CSF levels.

Several studies investigated the effects of intravenously applied SP in healthy human volunteers. Chiodera and Coira[110] and Coiro et al.,[111-114] for example, observed the effects of diurnal systemic administration of SP on the endocrine response in healthy young volunteers. SP was infused intravenously in doses of 0.5–1.5 pmol/kg/min (for 60 minutes) and alterations in plasma levels of the following hormones were evaluated: vasopressin, oxytocin,[110] growth hormone,[111] luteinising hormone, follicle-stimulating hormone (FSH),[112] thyroid-stimulating hormone (TSH),[113] adrenocorticotropic hormone (ACTH) and cortisol.[114] No significant side effects or changes in blood pressure were observed during SP infusions. While the lowest dose of SP had no effect on any of the hormones, SP 1.5 pmol/kg/min stimulated the secretion of vasopressin, growth hormone, luteinising hormone, ACTH and cortisol. The levels of oxytocin, FSH and TSH, however, remained unaffected. The pretreatment with sodium valproate completely abolished both ACTH and cortisol responses to SP.[114]

In a randomised, double-blind study in 12 healthy, young men,[115] we investigated the effect of nocturnal SP infusions (3 pmol/kg/min) on sleep, mood and neuroendocrine parameters. At this dose, side effects such as elevation of pulse rate, systolic blood pressure and flushing were observed but well tolerated by the subjects. As with Coiro et al., we found an increase in cortisol levels. In addition, TSH levels were increased, but GH remained unchanged. Moreover, SP infusions caused an immediate worsening of mood (feeling unhappy, melancholic, depressed) and a significant decrease of sleep quality, both of which might be caused by direct central effects of SP. However, it cannot be fully excluded that the results found are the mere consequences of the subjects' feeling stressed as a result of peripheral side effects during SP infusion.

3.2.5 The NK1R in Post-Mortem Brains of Psychiatric Patients

Burnet and Harrison[116] measured SP binding sites in the cingulate cortex in post-mortem brains of patients with unipolar depression (n = 13), bipolar disorder (n = 13), schizophrenia (n = 14) and controls (n = 14). In all brains, the authors found a higher density of SP binding sites in the superficial layers (laminae I–III) compared with deeper layers (laminae Va–VI) but no differences in overall binding site densities between the four groups. In unipolar depressed patients, however, a relative decrease of SP binding sites in superficial laminae compared with deep laminae could be found.

Stockmeier et al.,[117] investigating the density of SP binding sites in the post-mortem orbitofrontal cortex (BA 47) of 12 subjects with depression and 11 controls, found decreased binding sites in depressed patients across all cortical layers.

The mechanism involved in the apparent downregulation of NK1R in depression is not clear. Stockmeier et al.,[117] however, suggest that the decrease in NK1R in depression could represent an insufficient state-dependent adaptive alteration.

4. Substance P Receptor Antagonists (SPAs)

The fact that the SP/NK1R system has been proposed to be involved in the pathophysiology of a diverse range of conditions (see section 3) has opened up a wide array of potential indications for NK1R antagonists (substance P receptor antagonists [SPAs]), which fuelled major pharmaceutical research efforts starting in the early 1990s. Since the description of the first non-peptide SPA (CP-96345) by Snider et al.[118] in 1991, a plethora of highly selective, high-affinity, brain-penetrant, non-peptide SPAs have been produced.

Since it had been observed that neuropeptides are released preferentially when neurons are strongly activated and/or under pathological conditions, the idea was that peptide receptor antagonists only have an effect in deranged systems with increased neuropeptide levels, which might also lead to less pronounced side effects. In addition, because of the

modulatory nature of neuropeptides, it was speculated that the blockade of their receptors would show less dramatic effects than the antagonism at classic transmitter sites. Thus, the new class of antagonists may not only be suitable drugs but, in at least some aspects, even advantageous over established psychotropic drugs.[12]

SPAs were initially evaluated in the treatment of pain because of the well characterised role of SP in nociception (for review see Snijdelaar et al.[119]). However, SPAs such as aprepitant (MK-869, see figure 1), CP-99994, CP-122721, lanepitant (LY-303870), RP-100893, L-758298 or vofopitant (GR-205171) showed no, or only little, effect in the treatment of pain, including dental pain, migraine, osteoarthritis or peripheral neuropathy.[56,120] Similarly, an 8-week randomised, double-blind, placebo-controlled study conducted by Littman et al.[121] showed no effects on pain ratings, anxiety or depression in 30 fibromyalgia syndrome patients given the SPA ezlopitant (CJ-11974). The only significant effect was an improvement of dysesthesia in feet and hands.

To date, SPAs are developed as anxiolytics/antidepressants, which will be the focus of the following section. An additional field of development is in the treatment of chemotherapy-induced nausea and vomiting (CINV). SPAs block emesis in animal models and prevent acute and delayed CINV in the clinic.[56,122,123] In 2003, the US FDA approved the SPA aprepitant for the latter indication. Aprepitant is currently the first and only member of the SPA family to be authorised for clinical use.

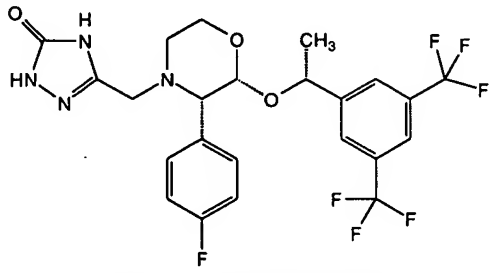

Fig. 1. Chemical structure of aprepitant (MK-869).

5. The Rationale for Developing SPAs for the Treatment of Affective and Anxiety Disorders

As described in section 2 and section 3.1.1, SP and NK1R are expressed in brain regions that are involved in the regulation of the stress/fear response as well as affective behaviour (e.g. amygdala, hippocampus, hypothalamus, frontal cortex), and the SP content in these areas changes upon application of stressful stimuli. In preclinical studies, the central administration of SP produces a range of fear-related behaviours. In addition, the SP/NK1R system shows significant spatial overlap with that of neurotransmitters such as serotonin (dorsal raphe nucleus) and norepinephrine (locus coeruleus), which are known to be involved in the regulation of mood, stress and anxiety. These findings have led to the suggestion that: (i) SP/NK1R pathways might play an important role in the modulation of stress-related, affective and/or anxious behaviour; and (ii) the blockade of NK1R might have anxiolytic as well as antidepressant effects.

5.1 Possible Mechanisms of Action of SPAs

On a molecular level, nonpeptide antagonists function by altering the general structure of the NK1R and by shifting it away from the agonist-binding conformation. Thus, SP and SPAs compete for occupancy of the receptor by binding in a mutually exclusive manner to distinct sites.[124]

On a functional level, several mechanisms of modulating affective and anxious behaviour have been suggested for SPAs. While it has been proposed that the SP/NK1R system and therefore SPAs may act via direct modulation of mood/behavioural responses to stressful stimuli, i.e. independently of other neurotransmitter pathways, an increasing number of studies suggest that the effect of SPAs may involve complex interactions with the hypothalamus-pituitary adrenal axis,[58,115] neurotransmitter systems and/or neurogenesis. These mechanisms will be discussed in detail in the following two sections.

5.1.1 Interactions of SPAs with Monoaminergic Systems

The pronounced anatomical overlap of the SP/NK1R and the monoaminergic neurotransmitter sys-

Table IV. Effects of genetic or pharmacological inactivation of neurokinin (NK)-1 receptor (NK1R) on noradrenaline (norepinephrine) and serotonin transmission

Species	Mode of NK1R inactivation	Effect	References
Guinea-pig	Acute/chronic L-760735	Increased firing of DRN neurons No desensitisation of 5-HT_{1A} autoreceptors	125
Guinea-pig	Acute/chronic L-760735	Acute: no effect on firing of LC neurons Chronic: increased burst firing of LC neurons No desensitisation of α_2-autoreceptors	126
Guinea-pig	Acute vofopitant (GR-205171)	Unchanged efflux of NA in FCx Blockade of SP-induced increase of NA efflux in FCx	127
Rat	Acute WIN-51, 708, CP-96, 345	No effect on firing of LC or DRN neurons Desensitisation of α_2-autoreceptors but unchanged sensitivity of 5-HT_{1A} autoreceptors	128
Rat	Subacute/chronic CP-96, 345	Increased firing of DRN neurons Desensitisation of 5-HT_{1A} autoreceptors	129
Rat	Acute vofopitant	Increased firing of LC neurons Increased efflux of NA in FCx and hippocampus, 5-HT efflux unchanged	130
Rat	Vofopitant	Unchanged efflux of 5-HT in hippocampus	22
Mouse	NK1R-/-[a] or acute RP-67580	Increased firing of DRN neurons In NK1R-/-: desensitisation of 5-HT_{1A} autoreceptors	131
Mouse	NK1R-/-	Unchanged basal firing of DRN neurons Unchanged basal efflux of 5-HT in FCx Increased effect of paroxetin on 5-HT efflux Desensitisation of 5-HT_{1A} autoreceptors	132
Mouse	Acute vofopitant	Unchanged efflux of 5-HT and NA in FCx	133

a NK1R-/- is genetically inactivated, i.e. 'knockout'.

5-HT = serotonin; **DRN** = dorsal raphe nucleus; **FCx** = frontal cortex; **LC** = locus coeruleus; **NA** = noradrenaline (norepinephrine).

tems, the well established role of the latter in modulation of affective behaviour, and the fact that alterations in serotonin and norepinephrine are believed to underlie the effects of many established psychotropic drugs, have raised extensive research into the interactions of SPAs with serotonin and norepinephrine systems. Table IV shows a summary of the effects of genetic or pharmacological inactivation of the NK1R on serotonin and norepinephrine transmission. NK1R inactivation seems to produce alterations in serotonin and norepinephrine neuronal activity that are likely to contribute to the antidepressant/anxiolytic efficacy of SPAs, but that are – at least partially – distinct from those produced by established drugs. This underlines the possible unique mechanism of action of this new class of compounds.

5.1.2 Effects of Genetic or Pharmacological NK1R Inactivation on Hippocampal Neurogenesis

Recent studies have shown that long-term antidepressant treatment not only leads to altered monoaminergic transmission, but also may result in increased hippocampal neurogenesis[134] and raised levels of brain-derived neurotrophic factor (BDNF).[135] In addition, Shirayama et al.,[136] using behavioural animal models of depression, found antidepressant effects of intrahippocampal injections of BDNF, whilst BDNF has been reported to increase basal levels of neurogenesis in the hippocampus.[137] Therefore, it has been suggested that antidepressants may ameliorate the symptoms of depression by modulating hippocampal neurogenesis[138] and by preventing or reversing the loss of hippocampal volume, which has been found in depressed patients.[139]

Psychosocial stress in tree shrews is currently being used as an animal model of depression.[140] Upon stress exposure, these animals also show reduced hippocampal neurogenesis and volume, both of which are reversed by antidepressant treatment.[141] In the same kind of model, van der Hart et al.[142] showed that comparable results can be obtained by long-term administration of an SPA and, in a very recent study, it was demonstrated that the hippocampi of NK1R knockout (NK1R-/-) mice (see section 5.2) contain significantly elevated

levels of BDNF and show increased neurogenesis.[143] These results not only suggest an involvement of SP/NK1R in hippocampal neurogenesis, but also a potential involvement of hippocampal neurogenesis in the mechanism of action of SPAs.

5.2 Preclinical Studies with SPAs

As mentioned previously (see section 2), the NK1R shows marked species variations in its amino acid sequence, which determines the species-dependent differences in the potency of various SPAs.[58] Since the majority of available SPAs have only reduced affinity for the rat and mouse NK1R – the most commonly used species in preclinical assays – it has become necessary to also evaluate SPA actions in species with human-like receptor pharmacology, such as gerbils, guinea-pigs and hamsters. Table V shows a summary of the effects of SPA administration on animal behaviour in behavioural models frequently used for assessing the activity of antidepressant and/or anxiolytic drugs. These assays are not intended to be models of depressive or anxiety disorders, but they provide useful means of comparing the actions of SPAs with established antidepressants/anxiolytics. To sum up, SPAs attenuate stress-induced behavioural defence responses and display anxiolytic- and antidepressant-like activity in certain experimental models. It should be noted that, unlike established anxiolytics and antidepressant drugs, SPAs did not cause motor impairment or sedation in the tested animals (with the exception of the light/dark shuttle box experiment by Zernig et al.[144]).

Another way of modelling a chronic blockade of NK1R is the creation of mice in which the NK1R has been genetically disrupted: the so-called 'NK1R knockout (NK1R-/-) mice'. Table VI summarises the phenotype of NK1R-/- mice in various behavioural paradigms. Except for some disaccord regarding the elevated plus-maze and the open-field, which might be due to different genetic background strains, there exists a general consensus that the NK1R-/- phenotype resembles that seen in wildtype mice treated with established antidepressant/anxiolytic drugs. In addition, there is generally good agreement between the effects of pharmacological blockade and genetic deletion of the NK1R on behaviour.

The marble-burying behaviour test, in which the burying of harmless objects (marbles) might reflect a form of impulsive behaviour, has been suggested to be a model for evaluating anti-obsessive-compulsive disorder drugs.[161] In a study conducted by Millan et al.,[162] the SPAs GR205171 and RP67580, by analogy with the SSRI fluvoxamine and the TCA clomipramine, completely blocked the marble-burying behaviour in mice.

The goal of further studies now is to gain more information about the location of the effects of the NK1R in certain behaviours. One way of approaching this task is by ablating the suspected region. For example, ablation of NK1R-expressing neurons in the amygdala, via local injections of the neurotoxin SP-saporin, reduced morphine reward behaviour and caused an increase in anxiety-like behaviours in the elevated plus-maze.[163] Rupniak et al.[148] showed that lesions of the basolateral amygdala (with ibotenic acid) inhibit fear conditioning in gerbils in the four plate test. Thus, the effect of amygdala lesions on fear conditioning resembles that seen after administration of the SPA L-760735 (see table V). These findings support the hypothesis that the amygdala, together with its associated output pathways, is one of the potential sites where SPAs may act to attenuate stress responses.[69]

All in all, these results provide further evidence that the SP/NK1R system is highly involved in the regulation of behavioural responses to stressful stimuli.

5.3 Clinical Studies with SPAs

The first clinical study that showed antidepressant as well as anxiolytic effects of an SPA was the study published in 1998 by Kramer et al.[22] The authors found that the SPA aprepitant showed improvements in depression and anxiety symptoms that were quantitatively comparable to those seen with paroxetine, an established SSRI, and significantly greater than those seen with placebo. The authors tested aprepitant in a randomised, double-blind, placebo-controlled multicentre study with 213 depressed outpatients. Anxiety levels of these depressed patients were moderately high. The patients were randomly assigned to daily treatment with aprepitant 300mg, paroxetine 20mg or placebo. They were treated for 6 weeks and primary outcome

Table V. Effects of substance P (SP) receptor antagonist (SPA) administration on animal behaviour in assays for antidepressant and anxiolytic drugs

Species	Injected SPA	Locus of injection	Effect	References
Guinea-pig	L-733060, L-760735, aprepitant (MK-869)	Systemic	Inhibition/attenuation of distress vocalisation (induced by NK1R-agonist or maternal separation)	22
Gerbil	L-733060, L-760735, aprepitant	Systemic	Inhibition of NK1R-agonist-induced foot tapping	22
Guinea-pig or mouse[a]	CP-99994, L-733060, vofopitant (GR-205171)	Systemic	Inhibition/attenuation of distress vocalisation (induced by NK1R-agonist or maternal separation)	145
Guinea-pig	L-760735	Amygdala	Attenuation of distress vocalisation (induced by maternal separation)	146
Gerbil	Aprepitant, CP-99994	Systemic	Inhibition of NK1R-agonist- or shock-induced foot tapping	147
Gerbil	L-760735	Systemic	Inhibition of NK1R-agonist-induced foot tapping, inhibition of fear conditioning	148
Hamster	L-760735	Systemic	Reduced aggression in RIT	149
Gerbil	L-760735	Systemic	Inhibition of NK1R-agonist-induced foot tapping, no effect in TST	149
Guinea-pig	L-760735	Systemic	No effect in EPM	149
Mouse	Vofopitant	Systemic	Inhibition of NK1R-agonist-induced grooming Reduced aggression RIT[b] Increased struggle behaviour in FST[a] No effect in TST	149
Rat	Vofopitant	Systemic	Inhibition of NK1R-agonist-induced sniffing No effect in EPM	149
Mouse	RP-67580	Systemic	Reduced anxiety-related behaviour in EPM, attenuation of distress vocalisation (induced by maternal separation)	131
Rat	NKP-608	Systemic	Anxiolytic effects in social interaction test of anxiety	150
Gerbil	L-760735, NKP-608	Systemic	Inhibition of NK1R-agonist-induced foot tapping Increased social interaction	151,152
Rats	FK-888	Lateral septal nucleus	Inhibition of anxiogenic-like response in EPM induced by ICV SP-injections	153
Rats	'Compound A'	Medial amygdala	Inhibition of anxiogenic-like profile induced by SP or immobilisation stress in EPM	69
Rats	NKP-608	Systemic	Increased sucrose intake in the chronic mild stress model of depression	154
Mouse	FK-888	ICV	Anxiolytic-like profile in EPM Inhibition of anxiogenic-like profile induced by SP or swim stress in EPM Inhibition of immunomodulatory effects induced by swim stress	74,155

Continued next page

Table V. Contd

Species	Injected SPA	Locus of injection	Effect	References
Mouse	Vofopitant	Systemic	Increased struggle behaviour in FST	133
Mouse	CP-96345	Systemic	Increased time spent in the more aversive light section of the LDSB (but also dose-dependent sedation and motor impairment)	144
Gerbil	Aprepitant, L-742694, L-733060, CP-99994, CP-122721	Systemic	Attenuation of NK1R-agonist induced foot tapping; anxiolytic-like profile in EPM	156
Rat	CP-96345, CP-99994	Caudal pontine reticular nucleus	Blocked sensitisation of the acoustic startle response	75
Cats	CP-96345	Systemic or in hypothalamus	Decrease/blockade of medial amygdaloid-induced facilitation of defensive rage	157

a Marginal enantioselectivity.

b Not enantioselective.

EPM = elevated plus-maze; **FST** = forced swim test; **ICV** = intracerebroventricular; **LDSB** = light/dark shuttle-box; **NK1R** = neurokinin-1 receptor; **RIT** = resident-intruder test; **TST** = tail suspension test.

measures were the Hamilton Depression Scale (HADS) and the Hamilton Anxiety Scale (HAM-A), as well as the Clinical Global Impressions-Severity Scale (CGI-S). Regarding symptoms of depression, as of week 1 and 2, aprepitant and paroxetine were more effective than placebo: 54% of the patients treated with aprepitant compared with 46% treated with paroxetine and 28% treated with placebo responded to the treatment, and 43% of the patients treated with aprepitant compared with 33% treated with paroxetine and 17% with placebo had a complete remission of depression (HADS <10). In addition, as of week 4 of the study, patients treated with aprepitant showed fewer symptoms of anxiety than patients in the placebo group, an effect that continued to increase through week 6. The authors suggested that the differential time course of the antidepressant and the anxiolytic effects might point to differing underlying mechanisms. All in all, aprepitant was very well tolerated: side effects did not occur more often than with placebo treatment. This was also the case for sexual dysfunction, which, in contrast, occurred in 26% of the patients treated with paroxetine and in 3% of patients treated with aprepitant. Treatment with aprepitant was not stopped more often than with placebo.

In a subsequent dose-finding study[56] conducted by the same authors with more than 800 depressed patients, the antidepressant efficacy of aprepitant 10mg, 20mg, 100mg and 300mg once daily and the active control, the SSRI fluoxetine 20mg once daily, was not superior to placebo treatment (discussed in Enserink[164]). Positron emission tomography imaging studies in human volunteers with the brain-penetrant tracer [18F]SPA-RQ suggest that very high levels of central NK1R occupancy (≥90%) are necessary to achieve therapeutically significant antidepressant effects. This criterion is fulfilled by an aprepitant dose of 300mg.[165] The fact that in the dose-finding study,[56] the reference drug fluoxetine did not differentiate from placebo, made the outcome of this study uninformative, a phenomenon that has been reported in approximately 50% of trials with various antidepressants.[166] However, Rupniak and Kramer[56] commented on this unpublished study, that *post hoc* analyses had shown antidepressant efficacy of aprepitant in the subset of severely depressed patients (HADS >25). Therefore,

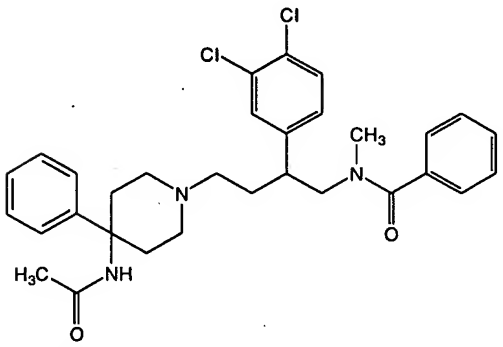

Fig. 2. Chemical structure of saredutant (SR-48968).

investigators conducted a further phase II trial, this time studying the efficacy of the SPA L-759274 (40 mg/day orally for 6 weeks) in 128 severely depressed patients (mean HADS = 28) with moderately high anxiety levels (mean HAM-A = 25) in a randomised, double-blind, placebo-controlled study. As in the initial aprepitant study of 1998,[22] SPA treatment was significantly superior to placebo treatment as assessed by HADS, HAM-A and CGI-S. L-759274 was generally well tolerated. The incidence of sexual side effects was on a par with that observed in patients receiving placebo, and the incidence of gastrointestinal effects was low.[167,168]

Another clinical trial, conducted with the SPA CP-122721, once more confirmed the antidepressant efficacy of SPAs: CP-122721 showed similar efficacy when compared with fluoxetine in depressed patients, and in a trial comparing CP-122721 to paroxetine and placebo, the drug exhibited fewer sexual side effects.[169]

To sum up, in three independent clinical trials with three different compounds, an antidepressant efficacy of SPAs could be demonstrated (specifically in severely depressed patients). In addition, SPAs showed significant anxiolytic effects in depressed patients. However, several more recent phase III trials, including relapse prevention studies conducted with aprepitant, failed to replicate the antidepressant effects as compared with control,[170] which led to a closing of the SPA antidepressant development programme of the respective company in autumn 2003. Nevertheless, several other pharmaceutical companies are currently conducting phase I and II trials with their structurally different and possibly more potent SPAs to assess their anxiolytic as well as antidepressant efficacies.

There are numerous indications from preclinical studies[74,127,171-173] that, in addition to SP and NK1R, other neurokinins and/or neurokinin receptors might also be involved in the modulation of stress-related behaviour and that exclusive blockade of the NK1R might not be sufficient to produce consistent anxiolytic and antidepressant effects. Sanofi-Aventis, for example, is currently conducting a clinical trial to assess the anxiolytic and antidepressant activity of the NK2R antagonist saredutant (SR-48968, figure 2).[174] Of special interest might also be substances that block more than one receptor type, such as NK1/2R antagonists or NK1/2/3R antagonists, which have been developed by certain companies.[175] These compounds may be more efficacious in antagonising the effects of SP than compounds that only block the NK1R.

Table VI. Behavioural differences in the phenotype of neurokinin (NK)-1 receptor (NK1R) knockout (NK1R-/-) mice as compared with their wildtype counterparts

Behavioural paradigm	NK1R-/- behavioural phenotype	References
Open-field	No difference	158
	Increased exploratory behaviour (nose pokes)	159
Elevated plus-maze	No difference	149,160
	Fewer anxiety-related behaviours	131
Resident-intruder	Less aggressive	149,158
Separation-induced vocalisation	Attenuated neonatal vocalisation	131,145
Novelty suppressed feeding	Fewer anxiety-related behaviours	131
Forced swim test	Increased struggle behaviour	149,159
Tail suspension test	Increased struggle behaviour	149
Others	Loss of the rewarding effect of morphine, and reduced response to opiate withdrawal	160
	Lower corticosterone levels after exposure to elevated plus-maze	131

6. Conclusion

Although contradictory results have been obtained in several studies, there is ample evidence to support the involvement of SP/NK1R in the regulation of the stress/fear response in animals and possibly in the modulation of depression and anxiety in humans. The proof of concept has been provided for NK1R antagonists in the treatment of major depression, but recent studies have challenged the usefulness of NK1R antagonists in the treatment of affective disorders. We believe that the potential for NK receptor antagonists as novel antidepressant and/or anxiolytic agents remains promising but needs further study, especially with respect to NK2Rs and NK3Rs and their contribution to behavioural modulation.

Acknowledgements

Inga Herpfer is a research fellow of the 'Deutsche Forschungsgemeinschaft' (DGF). Klaus Lieb received a grant from Merck Sharp & Dohme to study basic aspects of NK-1-receptor antagonists.

References

1. Murray CJL, Lopez AD, editors. Summary: the global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Published by the Harvard School of Public Health on behalf of the World Health Organization and the World Bank. Cambridge, MA: Harvard University Press, 1996
2. Holsboer F. The role of peptides in treatment of psychiatric disorders. J Neural Transm Suppl 2003; 64: 17-34
3. Von Euler US, Gaddum JH. An unidentified depressor substance in certain tissue extracts. J Physiol (Lond) 1931; 72: 74-87
4. Chang MM, Leeman SE, Niall HD. Amino acid sequence of substance P. Nat New Biol 1971; 232: 86-7
5. Duffy RA, Hedrick JA, Randolph G, et al. Centrally administered hemokinin-1 (HK-1), a neurokinin NK1 receptor agonist, produces substance P-like behavioral effects in mice and gerbils. Neuropharmacology 2003; 45: 242-50
6. Kurtz MM, Wang R, Clements MK, et al. Identification, localization and receptor characterization of novel mammalian substance P-like peptides. Gene 2002; 296: 205-12
7. Krause JE, Chirgwin JM, Carter MS, et al. Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. Proc Natl Acad Sci U S A 1987; 84: 881-5
8. Harmar AJ, Hyde V, Chapman K. Identification and cDNA sequence of delta-preprotachykinin, a fourth splicing variant of the rat substance P precursor. FEBS Lett 1990; 275: 22-4
9. Lai JP, Douglas SD, Rappaport E, et al. Identification of a delta isoform of preprotachykinin mRNA in human mononuclear phagocytes and lymphocytes. J Neuroimmunol 1998; 91: 121-8
10. Kotani H, Hoshimaru M, Nawa H, et al. Structure and gene organization of bovine neuromedin K precursor. Proc Natl Acad Sci U S A 1986; 83: 7074-8
11. Zhang Y, Lu L, Furlonger C, et al. Hemokinin is a hematopoietic-specific tachykinin that regulates B lymphopoiesis. Nat Immunol 2000; 1: 392-7
12. Hökfelt T, Broberger C, Xu ZQ, et al. Neuropeptides: an overview. Neuropharmacology 2000; 39: 1337-56
13. Pelletier G, Steinbusch HW, Verhofstad AA. Immunoreactive substance P and serotonin present in the same dense-core vesicles. Nature 1981; 293: 71-2
14. Pickel VM, Joh TH, Reis DJ, et al. Electron microscopic localization of substance P and enkephalin in axon terminals related to dendrites of catecholaminergic neurons. Brain Res 1979; 160: 387-400
15. Thureson-Klein A, Klein RL, Zhu PC. Exocytosis from large dense cored vesicles as a mechanism for neuropeptide release in the peripheral and central nervous system. Scan Electron Microsc 1986; (Pt 1): 179-87
16. Huang LYM, Neher E. Ca2+-dependent exocytosis in the somata of dorsal root ganglion neurons. Neuron 1996; 17: 135-45
17. Regoli D, Boudon A, Fauchere JL. Receptors and antagonists for substance P and related peptides. Pharmacol Rev 1994; 46: 551-99
18. Gether U, Johansen TE, Snider RM, et al. Different binding epitopes on the NK1 receptor for substance P and non-peptide antagonist. Nature 1993; 362: 345-8
19. Mantyh PW, Allen CJ, Ghilardi JR, et al. Rapid endocytosis of a G protein-coupled receptor: substance P evoked internalization of its receptor in the rat striatum in vivo. Proc Natl Acad Sci U S A 1995; 92: 2622-6
20. Grady EF, Garland AM, Gamp PD, et al. Delineation of the endocytic pathway of substance P and its seven-transmembrane domain NK1 receptor. Mol Biol Cell 1995; 6: 509-24
21. Smith DW, Hewson L, Fuller P, et al. The substance P antagonist L-760,735 inhibits stress-induced NK(1) receptor internalisation in the basolateral amygdala. Brain Research 1999; 848: 90-5
22. Kramer MS, Cutler N, Feighner J, et al. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 1998; 281: 1640-5
23. Takayama H, Ota Z, Ogawa N. Effect of immobilization stress on neuropeptides and their receptors in rat central nervous system. Regul Pept 1986 Oct; 15: 239-48
24. Chubb IW, Hodgson AJ, White GH. Acetylcholinesterase hydrolyzes substance P. Neuroscience 1980; 5: 2065-72
25. Persson S, Le Greves P, Thornwall M, et al. Neuropeptide converting and processing enzymes in the spinal cord and cerebrospinal fluid. Prog Brain Res 1995; 104: 111-30
26. Mai JK, Stephens PH, Hopf A, et al. Substance P in the human brain. Neuroscience 1986; 17: 709-39
27. Pioro EP, Mai JK, Cuello AC. Distribution of substance P and enkephalin immunoreactive neurons and fibers. In: Paxinos G, editor. The human nervous system. San Diego (CA): Academic Press, 1990: 1051-94
28. Nomura H, Shiosaka S, Tohyama M. Distribution of substance P-like immunoreactive structures in the brainstem of the adult human brain: an immunocytochemical study. Brain Res 1987; 404: 365-70
29. Chawla MK, Gutierrez GM, Young III WS, et al. Localization of neurons expressing substance P and neurokinin B gene transcripts in the human hypothalamus and basal forebrain. J Comp Neurol 1997; 384: 429-42
30. Feuerstein TJ, Gleichauf O, Landwehrmeyer GB. Modulation of cortical acetylcholine release by serotonin: the role of substance P interneurons. Naunyn Schmiedebergs Arch Pharmacol 1996; 354: 618-26
31. Battaglia G, Rustioni A. Coexistence of glutamate and substance P in dorsal root ganglion neurons of the rat and monkey. J Comp Neurol 1988; 277: 302-12

32. De Biasi S, Rustioni A. Glutamate and substance P coexist in primary afferent terminals in the superficial laminae of spinal cord. Proc Natl Acad Sci U S A 1988; 85: 7820-4
33. Hökfelt T, Millhorn D, Seroogy K, et al. Coexistence of peptides with classical neurotransmitters. Experientia 1987; 43 (7): 768-80
34. Sergeyev V, Hökfelt T, Hurd Y. Serotonin and substance P coexist in dorsal raphe neurons of the human brain. Neuroreport 1999; 10: 3967-70
35. Nakaya Y, Kaneko T, Shigemoto R, et al. Immunohistochemical localization of substance P receptor in the central nervous system of the adult rat. J Comp Neurol 1994; 347: 249-74
36. Mantyh PW. Neurobiology of substance P and the NK1 receptor. J Clin Psychiatry 2002; 63 Suppl. 11: 6-10
37. Dietl MM, Palacios JM. Phylogeny of tachykinin receptor localization in the vertebrate central nervous system: apparent absence of neurokinin-2 and neurokinin-3 binding sites in the human brain. Brain Res 1991; 539: 211-22
38. Jordan D, Kermadi I, Rambaud C, et al. Regional distribution of substance P binding sites in the brainstem of the human newborn. Brain Res 1995; 695: 117-24
39. Caberlotto L, Hurd YL, Murdock P, et al. Neurokinin 1 receptor and relative abundance of the short and long isoforms in the human brain. Eur J Neurosci 2003; 17: 1736-46
40. Tooney PA, Au GG, Chahl LA. Localisation of tachykinin NK1 and NK3 receptors in the human prefrontal and visual cortex. Neurosci Lett 2000; 283: 185-8
41. Chen LW, Wei LC, Liu HL, et al. Noradrenergic neurons expressing substance P receptor (NK1) in the locus coeruleus complex: a double immunofluorescence study in the rat. Brain Res 2000; 873: 155-9
42. Ma QP, Bleasdale C. Modulation of brain stem monoamines and gamma-aminobutyric acid by NK1 receptors in rats. Neuroreport 2002; 13: 1809-12
43. Commons KG, Valentino RJ. Cellular basis for the effects of substance P in the periaqueductal grey and dorsal raphe nucleus. J Comp Neurol 2002; 447: 82-97
44. Bartho L, Holzer P. Search for a physiological role of substance P in gastrointestinal motility. Neuroscience 1985; 16: 1-32
45. Greeno EW, Mantyh P, Vercellotti GM, et al. Functional neurokinin 1 receptors for substance P are expressed by human vascular endothelium. J Exp Med 1993; 177: 1269-76
46. Maggi CA. The effects of tachykinins on inflammatory and immune cells. Regul Pept 1997; 70: 75-90
47. Herrero JF, Laird JM, Lopez-Garcia JA. Wind-up of spinal cord neurons and pain sensation: much ado about something? Prog Neurobiol 2000; 61: 169-203
48. Quartara L, Maggi CA. The tachykinin NK1 receptor. Part II: distribution and pathophysiological roles. Neuropeptides 1998; 32: 1-49
49. Nilsson J, von Euler AM, Dalsgaard CJ. Stimulation of connective tissue cell growth by substance P and substance K. Nature 1985; 315: 61-3
50. Iwasaki Y, Kinoshita M, Ikeda K, et al. Trophic effect of various neuropeptides on the cultured ventral spinal cord of rat embryo. Neurosci Lett 1989; 101: 316-20
51. De Felipe C, Pinnock RD, Hunt SP. Modulation of chemotropism in the developing spinal cord by substance P. Science 1995; 267: 899-902
52. Lieb K, Fiebich BL, Busse-Grawitz M, et al. Effects of substance P and selected other neuropeptides on the synthesis of interleukin-1 beta and interleukin-6 in human monocytes: a re-examination. J Neuroimmunol 1996; 67: 77-81
53. Lieb K, Fiebich BL, Berger M, et al. The neuropeptide substance P activates transcription factor NF-kappaB and kappaB-dependent gene expression in human astrocytoma cells. J Immunol 1997; 159: 4952-8
54. Richardson JD, Vasko MR. Cellular mechanisms of neurogenic inflammation. J Pharmacol Exp Ther 2002; 302: 839-45
55. Barker R. Substance P and neurodegenerative disorders: a speculative review. Neuropeptides 1991; 20: 73-8
56. Rupniak NMJ, Kramer MS. Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK1) antagonists. Trends Pharmacol Sci 1999; 20: 485-90
57. Herpfer I, Lieb K. Substance P and substance P receptor antagonists in the pathogenesis and treatment of affective disorders. World J Biol Psychiatry 2003; 4: 56-63
58. Rupniak NMJ. Elucidating the antidepressant actions of substance P (NK1 receptor) antagonists. Curr Opin Investig Drugs 2002; 3: 257-61
59. Bannon MJ, Deutch AY, Tam SY, et al. Mild footshock stress dissociates substance P from substance K and dynorphin from Met- and Leu-enkephalin. Brain Res 1986 Sep 3; 381: 393-6
60. Lisoprawski A, Blanc G, Glowinski J. Activation by stress of the habenulo-interpeduncular substance P neurons in the rat. Neurosci Lett 1981 Aug 7; 25: 47-51
61. Siegel RA, Düker EM, Fuchs E, et al. Responsiveness of mesolimbic, mesocortical, septal and hippocampal cholecystokinin and substance P neuronal systems to stress, in the male rat. Neurochem Int 1984; 6: 783-9
62. Siegel RA, Duker EM, Pahnke U, et al. Stress-induced changes in cholecystokinin and substance P concentrations in discrete regions of the rat hypothalamus. Neuroendocrinology 1987 Jun; 46: 75-81
63. Nakamura H, Moroji T, Nohara S, et al. Effects of whole-body vibration stress on substance P- and neurotensin-like immunoreactivity in the rat brain. Environ Res 1990 Aug; 52: 155-63
64. Brodin E, Rosen A, Schott E, et al. Effects of sequential removal of rats from a group cage, and of individual housing of rats, on substance P, cholecystokinin and somatostatin levels in the periaqueductal grey and limbic regions. Neuropeptides 1994 Apr; 26: 253-60
65. Rosen A, Brodin K, Eneroth P, et al. Short-term restraint stress and s.c. saline injection alter the tissue levels of substance P and cholecystokinin in the peri-aqueductal grey and limbic regions of rat brain. Acta Physiol Scand 1992 Nov; 146: 341-8
66. Chowdrey HS, Larsen PJ, Harbuz MS, et al. Endogenous substance P inhibits the expression of corticotropin-releasing hormone during a chronic inflammatory stress. Life Sci 1995; 57: 2021-9
67. Allen BJ, Rogers SD, Ghilardi JR, et al. Noxious cutaneous thermal stimuli induce a graded release of endogenous substance P in the spinal cord: imaging peptide action in vivo. J Neurosci 1997; 17: 5921-7
68. Vaupel R, Jarry H, Schlomer HT, et al. Differential response of substance P-containing subtypes of adrenomedullary cells to different stressors. Endocrinology 1988; 123: 2140-5
69. Ebner K, Rupniak NM, Saria A, et al. Substance P in the medial amygdala: emotional stress-sensitive release and modulation of anxiety-related behavior in rats. Proc Natl Acad Sci U S A 2004; 101: 4280-5
70. Elliott PJ. Place aversion induced by the substance P analogue, dimethyl-C7, is not state dependent: implication of substance P in aversion. Exp Brain Research 1988; 73: 354-6
71. Aguiar MS, Brandão ML. Effects of microinjections of the neuropeptide substance P in the dorsal periaqueductal grey on the behaviour of rats in the plus-maze test. Physiol Behavior 1996; 60: 1183-6
72. Aguiar MS, Brandão ML. Conditioned place aversion produced by microinjections of substance P into the periaqueductal grey of rats. Behav Pharmacol 1994; 5: 369-73
73. Gavioli EC, Canteras NS, De Lima TC. Anxiogenic-like effect induced by substance P injected into the lateral septal nucleus. Neuroreport 1999; 10: 3399-403

74. Teixeira RM, Santos AR, Ribeiro SJ, et al. Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze behavior in mice. Eur J Pharmacol 1996; 311: 7-14
75. Krase W, Koch M, Schnitzler HU. Substance P is involved in the sensitization of the acoustic startle response by footshocks in rats. Behav Brain Res 1994; 63: 81-8
76. Brent PJ, Johnston PA, Chahl LA. Increased plasma catecholamines and locomotor activity induced by centrally administered substance P in guinea-pigs. Neuropharmacology 1988; 27: 743-8
77. Piot O, Betschart J, Grall I, et al. Comparative behavioural profile of centrally administered tachykinin NK1, NK2 and NK3 receptor agonists in the guinea-pig. Brit J Pharmacol 1995; 116: 2496-502
78. Bristow LJ, Young L. Chromodacryorrhea and repetitive hind paw tapping: models of peripheral and central tachykinin NK1 receptor activation in gerbils. Eur J Pharmacol 1994; 253: 245-52
79. Van Wimersma Greidanus TB, Maigret C. Grooming behavior induced by substance P. Eur J Pharmacol 1988; 154: 217-20
80. Katz RJ. Central injection of substance P elicits grooming behavior and motor inhibition in mice. Neurosci Lett 1979; 12: 133-6
81. Gradin K, Qadri F, Nomikos GG, et al. Substance P injection into the dorsal raphe increases blood pressure and serotonin release in hippocampus of conscious rats. Eur J Pharmacol 1992; 218: 363-7
82. Unger T, Carolus S, Demmert G, et al. Substance P induces a cardiovascular defense reaction in the rat: pharmacological characterization. Circ Res 1988; 63: 812-20
83. Hasenöhrl RU, Gerhardt P, Huston JP. Positively reinforcing effects of the neurokinin substance P in the basal forebrain: mediation by its C-terminal sequence. Exp Neurol 1992; 115: 282-91
84. Nikolaus S, Huston JP, Hasenöhrl RU. Reinforcing effects of neurokinin substance P in the ventral pallidum: mediation by the tachykinin NK1 receptor. Eur J Pharmacol 1999; 370: 93-9
85. Hasenöhrl RU, Jentjens O, De Souza Silva MA, et al. Anxiolytic-like action of neurokinin substance P administered systemically or into the nucleus basalis magnocellularis region. Eur J Pharmacol 1998; 354: 123-33
86. Bilkei-Gorzo A, Racz I, Michel K, et al. Diminished anxiety- and depression-related behaviors in mice with selective deletion of the Tac1 gene. J Neurosci 2002 Nov 15; 22: 10046-52
87. Oblin A, Zivkovic B, Bartholini G. Involvement of the D-2 dopamine receptor in the neuroleptic-induced decrease in nigral substance P. Eur J Pharmacol 1984; 105: 175-7
88. Brodin K, Ogren SO, Brodin E. Clomipramine and clonazepam increase cholecystokinin levels in rat ventral tegmental area and limbic regions. Eur J Pharmacol 1994; 263: 175-80
89. Hamon M, Gozlan H, Bourgoin S, et al. Opioid receptors and neuropeptides in the CNS in rats treated chronically with amoxapine or amitriptyline. Neuropharmacology 1987; 26: 531-9
90. Brodin E, Ogren SO, Theodorsson-Norheim E. Effects of subchronic treatment with imipramine, zimelidine and alaproclate on regional tissue levels of substance P- and neurokinin A/neurokinin B-like immunoreactivity in the brain and spinal cord of the rat. Neuropharmacology 1987; 26: 581-90
91. Shirayama Y, Mitsushio H, Takashima M, et al. Reduction of substance P after chronic antidepressants treatment in the striatum, substantia nigra and amygdala of the rat. Brain Res 1996; 739: 70-8
92. Rosen A, Franck J, Brodin E. Effects of acute systemic treatment with the 5 HT-uptake blocker alaproclate on tissue levels and release of substance P in rat periaqueductal grey. Neuropeptides 1995; 28: 317-24
93. Walker PD, Riley LA, Hart RP, et al. Serotonin regulation of neostriatal tachykinins following neonatal 6-hydroxydopamine lesions. Brain Res 1991; 557: 31-6
94. Riley LA, Jonakait GM, Hart RP. Serotonin modulates the levels of mRNAS coding for thyrotropin-releasing hormone and preprotachykinin by different mechanisms in medullary raphe neurons. Brain Res Mol Brain Res 1993; 17: 251-7
95. Sartori SB, Burnet PW, Sharp T, et al. Evaluation of the effect of chronic antidepressant treatment on neurokinin-1 receptor expression in the rat brain. Neuropharmacology 2004; 46: 1177-83
96. Rimon R, Le Greves P, Nyberg F, et al. Elevation of substance P-like peptides in the CSF of psychiatric patients. Biol Psychiatry 1984; 19: 509-16
97. Heikkila L, Rimon R, Terenius L. Dynorphin A and substance P in the cerebrospinal fluid of schizophrenic patients. Psychiatry Res 1990; 34: 229-36
98. Berrettini WH, Rubinow DR, Nurnberger JIJ, et al. CSF substance P immunoreactivity in affective disorders. Biol Psychiatry 1985; 20: 965-70
99. Toresson G, Brodin E, Wahlström A, et al. Detection of N-terminally extended substance P, but not of substance P in human cerebrospinal fluid - quantitation with HPLC-RIA. J Neurochem 1988; 50: 1701-7
100. Martensson B, Nyberg S, Toresson G, et al. Fluoxetine treatment of depression. Acta Psychiatr Scand 1989; 79: 586-96
101. Ackenheil M. Genetics and pathophysiology of affective disorders: relationship to fibromyalgia. Z Rheumatol 1998; 57 Suppl. 2: 5-7
102. Russell IJ. The promise of substance P inhibitors in fibromyalgia. Rheum Dis Clin North Am 2002; 28: 329-42
103. Schedlowski M, Fluge T, Richter S, et al. Beta-endorphin, but not substance-P, is increased by acute stress in humans. Psychoneuroendocrinology 1995; 20: 103-10
104. Weiss DW, Hirt R, Tarcic N, et al. Studies in psychoneuroimmunology: psychological, immunological, and neuroendocrinological parameters in Israeli civilians during and after a period of Scud missile attacks. Behav Med 1996; 22: 5-14
105. Bondy B, Baghai TC, Minov C, et al. Substance P serum levels are increased in major depression: preliminary results. Biol Psychiatry 2003; 53: 538-42
106. Lieb K, Walden J, Grunze H, et al. Serum levels of substance P and response to antidepressant pharmacotherapy. Pharmacopsychiatry. In press
107. Deuschle M, Sander P, Herpfer I, et al. Substance P in serum and cerebrospinal fluid of depressed patients: no effect of antidepressant treatment. Psychiatry Res. In press
108. Freed AL, Audus KL, Lunte SM. Investigation of substance P transport across the blood-brain barrier. Peptides 2002; 23: 157-65
109. Clark JW, Senanayake PD, Solomon GD, et al. Substance P: correlation of CSF and plasma levels. Headache 1994; 34: 261-4
110. Chiodera P, Coiro V. Effects of intravenous infusion of substance P on arginine vasopressin and oxytocin secretion in normal men. Brain Res 1992; 569: 173-6
111. Coiro V, Volpi R, Capretti L, et al. Intravenously infused substance P enhances basal and growth hormone (GH) releasing hormone-stimulated GH secretion in normal men. Peptides 1992; 13: 843-6
112. Coiro V, Volpi R, Capretti L, et al. Luteinizing hormone response to an intravenous infusion of substance P in normal men. Metabolism 1992; 41: 689-91
113. Coiro V, Volpi R, Capretti L, et al. Effect of substance P on basal and thyrotropin-releasing hormone-stimulated thyrotropin release in humans. Metabolism 1995; 44: 474-7

114. Coiro V, Capretti L, Volpi R, et al. Stimulation of ACTH/cortisol by intravenously infused substance P in normal men: inhibition by sodium valproate. Neuroendocrinology 1992; 56: 459-63
115. Lieb K, Ahlvers K, Dancker K, et al. Effects of the neuropeptide substance P on sleep, mood, and neuroendocrine measures in healthy young men. Neuropsychopharmacol 27; 2002: 1041-9
116. Burnet PW, Harrison PJ. Substance P (NK1) receptors in the cingulate cortex in unipolar and bipolar mood disorder and schizophrenia. Biol Psychiatry 2000; 47: 80-3
117. Stockmeier CA, Shi X, Konick L, et al. Neurokinin-I receptors are decreased in major depressive disorder. Neuroreport 2002; 13: 1223-7
118. Snider RM, Constantine JW, Lowe IJ, et al. A potent nonpeptide antagonist of the substance P (NK1) receptor. Science 1991; 251: 435-7
119. Snijdelaar DG, Dirksen R, Slappendel R, et al. Substance P. Eur J Pain 2000; 4: 121-35
120. Hill R. NK1 (substance P) receptor antagonists - why are they not analgesic in humans? Trends Pharmacol Sci 2000; 21: 244-6
121. Littman BH, Newton FA, Russell IJ. Substance P antagonism in fibromyalgia: a trial with CJ-11974. In: Abstracts from the World Conference on Pain; Seattle, WA: IASP Press, 1999: 67
122. Van Belle S, Lichinitser MR, Navari RM, et al. Prevention of cisplatin-induced acute and delayed emesis by the selective neurokinin-1 antagonists, L-758,298 and MK-869. Cancer 2002; 94: 3032-41
123. Saito R, Takano Y, Kamiya HO. Roles of substance P and NK(1) receptor in the brainstem in the development of emesis. J Pharmacol Sci 2003; 91: 87-94
124. Quartara L, Maggi CA. The tachykinin NK1 receptor. Part I: ligands and mechanisms of cellular activation. Neuropeptides 1997; 31: 537-63
125. Conley RK, Cumberbatch MJ, Mason GS, et al. Substance P (neurokinin 1) receptor antagonists enhance dorsal raphe neuronal activity. J Neurosci 2002; 22: 7730-6
126. Maubach KA, Martin K, Chicchi G, et al. Chronic substance P (NK1) receptor antagonist and conventional antidepressant treatment increases burst firing of monoamine neurons in the locus coeruleus. Neuroscience 2002; 109: 609-17
127. Bert L, Rodier D, Bougault I, et al. Permissive role of neurokinin NK(3) receptors in NK(1) receptor-mediated activation of the locus coeruleus revealed by SR 142801. Synapse 2002; 43: 62-9
128. Haddjeri N, Blier P. Effect of neurokinin-1 receptor antagonists on the function of 5-HT and noradrenaline neurons. Neuroreport 2000; 11: 1323-7
129. Haddjeri N, Blier P. Sustained blockade of neurokinin-1 receptors enhances serotonin neurotransmission. Biol Psychiatry 2001; 50: 191-9
130. Millan MJ, Lejeune F, De Nanteuil G, et al. Selective blockade of neurokinin (NK)(1) receptors facilitates the activity of adrenergic pathways projecting to frontal cortex and dorsal hippocampus in rats. J Neurochem 2001; 76: 1949-54
131. Santarelli L, Gobbi G, Debs PC, et al. Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. Proc Natl Acad Sci U S A 2001; 98: 1912-7
132. Froger N, Gardier AM, Moratalla R, et al. 5-hydroxytryptamine (5-HT)1A autoreceptor adaptive changes in substance P(neurokinin 1) receptor knock-out mice mimic antidepressant-induced desensitization. J Neurosci 2001; 21: 8188-97
133. Zocchi A, Varnier G, Arban R, et al. Effects of antidepressant drugs and GR 205171, an neurokinin-1 (NK1) receptor antagonist, on the response in the forced swim test and on monoamine extracellular levels in the frontal cortex of the mouse. Neurosci Lett 2003; 345: 73-6
134. Manev H, Uz T, Smalheiser NR, et al. Antidepressants alter cell proliferation in the adult brain in vivo and in neural cultures in vitro. Eur J Pharmacol 2001; 411: 67-70
135. Chen B, Dowlatshahi D, MacQueen GM, et al. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 2001; 50: 260-5
136. Shirayama Y, Chen AC, Nakagawa S, et al. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 2002; 22: 3251-61
137. Lee J, Duan W, Mattson MP. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. J Neurochem 2002; 82: 1367-75
138. Santarelli L, Saxe M, Gross C, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003; 301: 805-9
139. Bremner JD, Narayan M, Anderson ER, et al. Hippocampal volume reduction in major depression. Am J Psychiatry 2000; 157: 115-8
140. van Kampen M, Kramer M, Hiemke C, et al. The chronic psychosocial stress paradigm in male tree shrews: evaluation of a novel animal model for depressive disorders. Stress 2002; 5: 37-46
141. Czeh B, Michaelis T, Watanabe T, et al. Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci U S A 2001; 98: 12796-801
142. van der Hart MG, Czeh B, de Biurrun G, et al. Substance P receptor antagonist and clomipramine prevent stress-induced alterations in cerebral metabolites, cytogenesis in the dentate gyrus and hippocampal volume. Mol Psychiatry 2002; 7: 933-41
143. Morcuende S, Gadd CA, Peters M, et al. Increased neurogenesis and brain-derived neurotrophic factor in neurokinin-1 receptor gene knockout mice. Eur J Neurosci 2003; 18: 1828-36
144. Zernig G, Dietrich H, Maggi CA, et al. The substance P (NK1) receptor antagonist (+/-)-CP-96,345 causes sedation and motor impairment in Swiss albino mice in the black-and-white box behavioral paradigm. Neurosci Lett 1992; 143: 169-72
145. Rupniak NM, Carlson EC, Harrison T, et al. Pharmacological blockade or genetic deletion of substance P (NK(1)) receptors attenuates neonatal vocalisation in guinea-pigs and mice. Neuropharmacology 2000; 39: 1413-21
146. Boyce S, Smith D, Carlson E, et al. Intra-amygdala injection of the substance P [NK(1) receptor] antagonist L-760735 inhibits neonatal vocalisations in guinea-pigs. Neuropharmacology 2001; 41: 130-7
147. Ballard TM, Sanger S, Higgins GA. Inhibition of shock-induced foot tapping behaviour in the gerbil by a tachykinin NK1 receptor antagonist. Eur J Pharmacol 2001; 412: 255-64
148. Rupniak NM, Webb JK, Fisher A, et al. The substance P (NK1) receptor antagonist L-760735 inhibits fear conditioning in gerbils. Neuropharmacology 2003; 44: 516-23
149. Rupniak NM, Carlson EJ, Webb JK, et al. Comparison of the phenotype of NK1R-/- mice with pharmacological blockade of the substance P (NK1) receptor in assays for antidepressant and anxiolytic drugs. Behav Pharmacol 2001; 12: 497-508
150. File SE. NKP608, an NK1 receptor antagonist, has an anxiolytic action in the social interaction test in rats. Psychopharmacology 2000; 152: 105-9
151. Cheeta S, Tucci S, Sandhu J, et al. Anxiolytic actions of the substance P (NK1) receptor antagonist L-760735 and the 5-HT1A agonist 8-OH-DPAT in the social interaction test in gerbils. Brain Res 2001; 915: 170-5
152. Gentsch C, Cutler M, Vassout A, et al. Anxiolytic effect of NKP608, a NK1-receptor antagonist, in the social investigation test in gerbils. Behav Brain Res 2002; 133: 363-8

153. Gavioli EC, Canteras NS, De Lima TCM. The role of lateral septal NK1 receptors in mediating anxiogenic effects induced by intracerebroventricular injection of substance P. Behav Brain Res 2002; 134: 411-5

154. Papp M, Vassout A, Gentsch C. The NK1-receptor antagonist NKP608 has an antidepressant-like effect in the chronic mild stress model of depression in rats. Behav Brain Res 2000; 115: 19-23

155. Teixeira RM, De Lima TC. Involvement of tachykinin NK1 receptor in the behavioral and immunological responses to swimming stress in mice. Neuropeptides 2003; 37: 307-15

156. Varty GB, Cohen-Williams ME, Morgan CA, et al. The gerbil elevated plus-maze II: anxiolytic-like effects of selective neurokinin NK1 receptor antagonists. Neuropsychopharmacology 2002; 27: 371-9

157. Shaikh MB, Steinberg A, Siegel A. Evidence that substance P is utilized in medial amygdaloid facilitation of defensive rage behavior in the cat. Brain Res 1993; 625: 283-94

158. De Felipe C, Herrero JF, O'Brien JA, et al. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature 1998; 392: 394-7

159. Santarelli L, Gobbi G, Blier P, et al. Behavioral and physiologic effects of genetic or pharmacologic inactivation of the substance P receptor (NK1). J Clin Psychiatry 2002; 63 Suppl. 11: 11-7

160. Murtra P, Sheasby AM, Hunt SP, et al. Rewarding effects of opiates are absent in mice lacking the receptor for substance P. Nature 2000; 405: 180-3

161. Takeuchi H, Yatsugi S, Yamaguchi T. Effect of YM992, a novel antidepressant with selective serotonin re-uptake inhibitory and 5-HT 2A receptor antagonistic activity, on a marble-burying behavior test as an obsessive-compulsive disorder model. Jpn J Pharmacol 2002; 90: 197-200

162. Millan MJ, Girardon S, Mullot J, et al. Stereospecific blockade of marble-burying behaviour in mice by selective, non-peptidergic neurokinin1 (NK1) receptor antagonists. Neuropharmacology 2002; 42: 677-84

163. Gadd CA, Murtra P, De Felipe C, et al. Neurokinin-1 receptor-expressing neurons in the amygdala modulate morphine reward and anxiety behaviors in the mouse. J Neurosci 2003; 23: 8271-80

164. Enserink M. Can the placebo be the cure? Science 1999; 284: 238-40

165. Hargreaves R. Imaging substance P receptors (NK1) in the living human brain using positron emission tomography. J Clin Psychiatry 2002; 63 Suppl. 11: 18-24

166. Quitkin FM, Rabkin JG, Gerald J, et al. Validity of clinical trials of antidepressants. Am J Psychiatry 2000; 157: 327-37

167. Ranga K, Krishnan R. Clinical experience with substance P receptor (NK1) antagonists in depression. J Clin Psychiatry 2002; 63 Suppl. 11: 25-9

168. Kramer MS, Winokur A, Kelsey J, et al. Demonstration of the efficacy and safety of a novel substance P (NK1) receptor antagonist in major depression. Neuropsychopharmacology 2004; 29: 385-92

169. Chappell P. Effects of CP122721, a selective NK1 receptor antagonist in patients with major depression. Presented at the 42nd annual meeting of the New Clinical Drug Evaluation Unit (NCDEU); 2002 Jun 12, Boca Raton (FL), USA

170. Montgomery SA, Keller M, Ball W, et al. Peptide approaches in the treatment of major depression: lack of efficacy of the substance P (neurokinin 1 receptor) antagonist aprepitant. Eur Neuropsychopharmacol 2004; 14 (Suppl. 3): S136-37

171. Steinberg R, Alonso R, Griebel G, et al. Selective blockade of neurokinin-2 receptors produces antidepressant-like effects associated with reduced corticotropin-releasing factor function. J Pharmacol Exp Ther 2001; 299: 449-58

172. Ribeiro SJ, Teixeira RM, Calixto JB, et al. Tachykinin NK(3) receptor involvement in anxiety. Neuropeptides 1999; 33: 181-8

173. Massi M, Panocka I, de Caro G. The psychopharmacology of tachykinin NK-3 receptors in laboratory animals. Peptides 2000; 21: 1597-609

174. Holmes A, Heilig M, Rupniak NM, et al. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol Sci 2003; 24: 580-8

175. Giardina GA, Gagliardi S, Martinelli M. Antagonists at the neurokinin receptors: recent patent literature. IDrugs 2003; 6: 758-72

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Current Pharmaceutical Design, 2005, *11*, 1529-1547

Do Substance P and the NK_1 Receptor have a Role in Depression and Anxiety?

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Abstract: Research on Substance P (SP) has, until recently, focused on its role in pain and inflammation. However, a report that NK_1 receptor antagonists have utility in the treatment of depression has stimulated research into the function of SP and the NK_1 receptor in anxiety and depression. The distribution of SP and the NK_1 receptor in brain areas implicated in anxiety and depression is initially reviewed. This is followed by evaluation of the preclinical data obtained for SP and NK_1 receptor antagonists in behavioral models of depression as well as the phenotype of genetically modified animals lacking the genes encoding for the NK_1 receptor or for SP. The weight of the evidence supports antidepressant and anxiolytic activity of NK_1 receptor antagonists. However, many of the studies do not control for nonspecific effects of the compounds, and when enantiomers that lack activity at the NK_1 receptor are included, the results, in some cases, suggest that blockade of NK_1 receptors does not account for the observed behavioral activity. Finally, clinical studies in depressed patients assessing SP levels in plasma and cerebrospinal fluid as well as the effect of NK_1 receptor antagonists are reviewed. The clinical studies are a mixture of positive, failed and negative studies on the antidepressant activity of NK_1 receptor antagonists, not unlike the early clinical results obtained with selective serotonin reuptake inhibitors.

Key Words: Substance P, NK_1 receptor, depression, anxiety, clinical studies.

INTRODUCTION

Substance P (SP) was first identified in 1931 by Von Euler and Gaddum and in 1970 the amino acid sequence of the undecapeptide was elucidated [1]. SP is derived from the preprotachykinin-A gene (PPT-A) through alternative splicing[2], binding preferentially to the tachykinin NK_1 receptor[3]. However, SP is but one member of the family of mammalian tachykinin peptides that also includes Neurokinin A (NKA) and Neurokinin B (NKB) (reviewed in Otsuka and Yoshioka [4]). Both SP and NKA are derived from the PPT-A gene. Alternative RNA splicing yields three precursors α-, β- and γ-PPT-A each containing a copy of SP while β- and γ- PPT-A encode both NKA and SP (Fig. 1). A separate gene, preprotachykinin B (PPT-B) encodes for NKB. The neurokinins all have in common the COOH terminal sequence Phe-X-Gly-Leu-Met NH_2 that is necessary but not sufficient for biological activity at their respective receptors.

SP binds relatively selectively to the NK_1 receptor while NKA is the preferred ligand for the NK_2 receptor and NKB for the NK_3 receptor (Table 1). This review concentrates on SP and the NK_1 receptor, however recent clinical trials as well as preclinical data suggest that both the NKA and NKB systems may play a role in psychiatric disease. Although the presence of NK_2 receptors in brain has been a matter of some debate. [5, 6], the NK_2 receptor antagonist Saredutant (SR48968) exhibits activity in antidepressant and anxiolytic models [7, 8] and is currently undergoing clinical evaluation in depressed patients. NK_1 receptors are widely distributed in brain [9-11] and have been shown to modulate the activity of dopaminergic, noradrenergic and cholinergic systems [12, 13]. NK_1 receptor antagonists block senktide (selective NK_3 agonist)- induced firing of dopamine containing cells as well as the turning behavior elicited by intrastriatal infusion of senktide [14, 15] focusing attention on NK_3 receptor antagonists as possible antipsychotic agents. In fact, the selective NK_3 receptor antagonists, osanetant (SR142801) and talnetant (SKD) have recently been reported on Sanofi's and Glaxo's Web sites, respectively to be active against schizophrenia in Phase II trials.

The focus of much preclinical work on SP has been on its role in the transmission of nociceptive information in contrast to "emotional" behavior. Yet, paradoxically, clinical trials with selective antagonists of the NK_1 receptor have failed to show any significant analgesic activity (see [16]) but have shown antidepressant activity.

Depression involves disruption of emotional and cognitive processes and thus the distribution of SP and NK_1 receptors in both man and rat are reviewed with a focus on brain circuits involved in emotional and cognitive processing. Evidence for anxiolytic and antidepressant activity of NK_1 receptor antagonists derived from behavioral experiments is assessed, concluding with the results of clinical trials evaluating the activity of NK_1 receptor antagonists in depression.

LOCALIZATION OF SUBSTANCE P AND NK_1 RECEPTOR IN BRAIN

The distribution of substance P and the NK_1 receptor in brain has been extensively reviewed previously [4, 17-19]. However, consideration of the distribution of both peptide and receptor in frontal cortex, amygdala, dorsal raphe and

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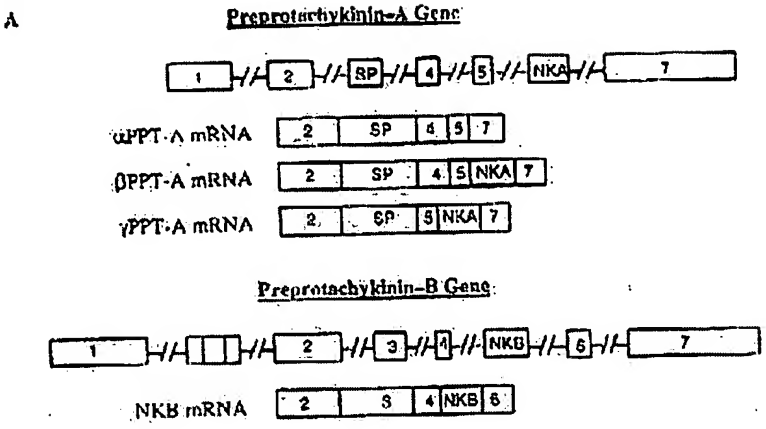

B

Primary Structure of Substance P and Related Peptides

Peptide	*Sequence*
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2

Fig. (1). Preprotachykinin genes, mRNA and peptide structures.

locus coeruleus is warranted as these areas are involved in processing emotional stimuli, responding to stress and have been shown to be altered in depression. Efforts will be made to highlight any species differences in the distribution of SP or NK_1 receptors.

Table 1. Tachykinin Receptors; Pharmacology and Localization

Receptor	Pharmacology	Location
NK_1	SP>NKA>NKB	Brain Glia Smooth Muscle Lymphocytes
NK_2	NKA>NKB>SP	Brain Smooth Muscle
NK_3	NKB>NKA>SP	Brain

FRONTAL CORTEX

Imaging, electrophysiological and lesion studies in man and animals point toward a role for the prefrontal cortex (PFC) in emotional behavior (see Drevets [20]). The PFC has been shown to alter fear behavior, participate in modulating autonomic and endocrine responses to stress and to exhibit altered metabolic activity in patients with major depression [21]. Reports of structural changes in the frontal cortex of depressed individuals [22] suggests that this is an area that may contribute to the etiology of depression and by inference the antidepressant activity of pharmacological agents.

In the prefrontal cortex of man, NK_1 receptors are found in superficial layers I-IV similar to their localization in rat frontal cortex [23-26]. Immunolocalization of the NK_1 receptor in rat indicates that the neocortical neurons expressing substance P receptor are GABAergic non-pyramidal cells, a mixed population of heavily stained, large multipolar cells scattered throughout the neocortical layers except for layer I and weakly immunoreactive medium sized multipolar cells distributed in all cortical layers with a bias to layers II–III and the superficial part of layer V [27]. Experiments in entorhinal cortex suggests that NK_1 receptor activation promotes the release of GABA at synapses on principal neurons [28].

The distribution of NK_1 receptor generally coincides with the distribution of SP fibers found in the cortical layers of both rat and man [17, 29]. In rat medial prefrontal cortex corresponding to the primate prelimbic area, large coarse SP containing fibers are present throughout layers II, III and IV[30]. Double labeling studies suggest the majority of SP positive cells also contain GABA and are likely to be part of

a local inhibitory circuit [31]. While many of the SP positive fibers are likely to represent axons from neurons intrinsic to the rat cortex, several studies have raised the possibility that some fibers originate outside of the cortex, perhaps from the lateral tegimental nucleus adjacent to the locus coeruleus [32, 33].

In primate frontal cortex, substance P –like immunoreactivity (SPLI) is biased toward the upper cortical layers in contrast to the rat which has a paucity of SPLI in layer I. In man, the greatest density of SP fibers as well as SP positive non-pyramidal bipolar cells, are found in layers II and IV of frontal cortex [29]. Fibers in the deep layers run perpendicular to the cortical surface while those in superficial layers proceed horizontally [34] suggesting that SP containing cells may be able to effect large areas of cortical functioning. The bipolar cells in man may be similar to the bipolar cells in the monkey prefrontal cortex that contain GABA and SP [35]. In contrast to the rat, there is no evidence for extrinsic SP containing fibers innervating the frontal cortex of primates [35, 36]. The SP containing interneurons synapse on pyramidal cells and on cortical interneurons consistent with SP playing an important role in the control of inhibitory cortical function. In rat entorhinal cortex, NK$_1$ receptor activation promotes the release of GABA at synapses on principal neurons [17] inhibiting pyramidal output neurons. Whether the GABAergic interneurons that are excited by SP are the same interneurons that colocalize SP and GABA is not clear. It is unknown whether these results in the entorhinal cortex can be generalized to the frontal cortex, but suggests further work localizing SP-/GABA and the NK$_1$ receptor is needed to understand the role of SP in the functioning of the frontal cortex circuitry.

While NK$_1$ receptors have been localized to GABA interneurons through which they inhibit the activity of pyramidal cells, NK$_1$ receptor activation has also been shown to excite cortical cells presumably through activation of glutamate containing excitatory interneurons [37]. Whether the different effects can be anatomically segregated as suggested by SP-induced depression of *in vivo* neuronal activity in the superficial layers, but excitation in deeper layers will need to be resolved [38].

In a series of studies relevant to antidepressant activity of NK$_1$ receptor antagonists in the cortex, the sensitivity of cingulate cortex neurons to iontophoretically applied SP following antidepressant treatment, activation of noradrenergic input and electro-convulsive shock (ECS) was examined. Iontophoretic application of SP elicited excitation of the majority of neurons in layers IV and V of the rat cingulate cortex. Following a 14 day course of ECS there was a marked reduction in the excitatory response to SP [39]. SP-elicited excitation of cortical cells was also reduced by iontophoretic application of norepinephrine or by stimulation of the locus coeruleus to activate noradrenergic input to the cingulate cortex [40, 41]. Conversely, lesions of the locus coeruleus increased the sensitivity of cingulate cells to SP [41]. Surprisingly, 24 to 36 hr after the last of 14 consecutive daily treatments with tranylcypromine, carbamazepine or oxaprotiline there was an increase in neuronal sensitivity to substance P with all three drugs. None of the compounds altered responses to substance P after a single acute treatment. Thus, ECS decreased but chronic antidepressant treatment increased excitatory responses to SP in the entorhinal cortex. Increased release of NE, following ECS and LC activation could underlie the dampening of SP-induced excitation. Paradoxically, MAO inhibitors would be expected to increase NE levels in frontal cortex [42], and thus decrease not increase sensitivity to SP-induced excitation. Thus, decreased SP responsivity frontal cortex does not appear to be a common mechanism underlying the antidepressant activity of ECS and MAO-inhibitors. If NE modulates the effect of SP on cortical neurons, it would be interesting to observe the effect of a selective norepinephrine reuptake inhibitor such as reboxetine.

In the Flinders Resistant line of rats, that model aspects of depression, several studies examined changes in NK$_1$ receptors as well as SP levels in the frontal cortex following antidepressant treatment [43]. Chronic electroconvulsive shock (ECS) elevated NK$_1$ receptor levels in rat cortex of rat when measured by autoradiography, however in the same study there was no change in NK$_1$ mRNA levels [44]. SP levels in frontal cortex have been reported to increase following ECS or administration of an SSRI [45]. However, others have not seen any change following ECS [46]. Finally, the Flinders line of rats, purported to express a "depressive phenotype", have been shown to have decreased levels of SP-like immunoreactivity in the frontal cortex that is normalized with chronic, but not acute treatment with lithium [47].

The observed elevation of both SP and NK$_1$ receptors in response to antidepressant and electroconvulsive shock treatment is difficult to reconcile with blockade of NK$_1$ receptors producing an antidepressant response. However, it is not clear whether the changes in SP and NK$_1$ receptor levels reflect increased or decreased neurotransmission. Examination of receptor internalization in the frontal cortex might help to shed light on this, as this method has been used to show that the SP receptor undergoes internalization following stimulation [48, 49].

If the decreased levels of SP in the Flinders line of rats reflect increased release of SP this would be consistent with a blockade of NK$_1$ receptors producing an antidepressant response. While lithium may normalize the levels of SP in the Flinders rats, lithium is not antidepressant and on its own has been shown to elevate levels of SP in the frontal cortex of Fischer rats [50]. The Flinders line of rats represents an interesting model of depression warranting further investigation of the role of SP in the "depressive phenotype". Would an antagonist with high affinity for the rodent NK$_1$ receptor reverse some of the behavioral deficits found in the Flinders rat?

Elucidating the conditions under which SP excites or inhibits the firing of principal cells of the cortex will be important. Speculatively, SP may have an excitatory action on inhibitory interneurons in the cortex, perhaps dampening output from the frontal cortex. Attenuation of the excitatory actions of SP by NE suggests that elevating noradrenergic tone in frontal cortex might result in disinhibition of the pyramidal neurons and increase excitatory output from the frontal cortex. This scenario would be consistent with the reduction in cerebral blood flow in frontal cortex observed in

depressed subjects [51] and its normalization with antidepressant treatment.

AMYGDALA

Anatomically the amygdala is part of a circuit that includes the prefrontal cortex and hippocampus and like the prefrontal cortex has been reported in depressed patients to have abnormalities of blood flow and glucose metabolism [21, 51]. The central, lateral, basal and accessory basal nuclei of the amygdala are thought to participate in a brain circuit that underlies emotion derived in part from their importance in the development of fear conditioning [52]. Additional support for this conceptualization comes from studies demonstrating that infusion of benzodiazepines into the amygdala has anxiolytic effects in a variety of tests [53-55] and similarly infusion of antidepressants into the amygdala elicits an antidepressant effect in the rat forced swim test [56].

The distribution of SP in the amygdala of rat has been extensively characterized [18, 57]. Cells immunoreactive for SP are present in the medial and to a lesser extent in the central nucleus of the amygdala [58]. A dense fiber plexus appears to originate in the medial nucleus and course into the central nucleus [59] as well as into the lateral and basolateral nuclei [57, 60-62]. Some of these SP positive fibers may represent an output pathway via the stria terminalis [63]. The distribution of NK_1 receptors within the amygdala also varies as a function of subnuclei. Consistent with the SP containing fibers coursing from medial to central nucleus, NK_1 receptors labeled by ^{3}H-SP and a large number of cells positive for mRNA encoding the NK_1 receptor are found in the central nucleus [25, 64]. Interestingly, a large number of cells expressing mRNA for NK_1 were reported present in the medial nucleus, however, receptor binding appeared low in the medial nucleus possibly reflecting transport of receptor to terminal regions outside of the medial nucleus.

Cells expressing peptide as well as mRNA encoding for SP are present in human amygdaloid nuclei [65-67]. Similar to the rat, the medial, central and cortical nuclei as well as the cortico-amygdalar-transition area exhibit a dense plexus of SP immunoreactive fibers [68]. Cells immunoreactive for SP are found throughout the amygdala with the highest numbers found in the cortical transition area, the central and cortical nuclei, while the lateral nucleus has few labeled neurons.

The amygdala is reported to have the fourth highest level of [^{125}I]BH SP binding in human brain exceeded only by the caudate, putamen and hypothalamus [19]. *In situ* hybridization and localization of mRNA encoding the NK_1 receptor reveals a moderate level of hybridization signal in the lateral and accessory basal nuclei with low levels in the basal nucleus. A uniform and moderate level of binding sites localized by [^{3}H] SP binding are observed in these same nuclei [69]. It is difficult to tell if there is a concordance of receptor and SP containing fibers in human amygdala, however this could be resolved by double labeling for receptor and peptide.

In the basolateral amygdala of guinea pig and human, NK_1 receptors are largely restricted to GABA containing interneurons similar to that observed in the frontal cortex [70]. In slices from guinea pig brains, activation of NK_1 receptors elicited inhibitory post synaptic potentials in the majority of basolateral projection neurons consistent with release of GABA [70]. However, employing *in vivo* recording from anesthetized rats, iontophoretic application of SP elicited a prolonged, but slow onset excitation of neurons in the medial nucleus of the amygdala [71]. The conflicting results are presumed to reflect different synaptic organization of the various amygdaloid nuclei, but species differences, as well as methodological differences inherent in *in vitro* and *in vivo* recordings cannot be ruled out.

Studies demonstrating a reduction in SP levels in the amygdala following chronic, but not acute antidepressant treatment [72] suggest a role for substance P in the antidepressant response although the relationship between altered levels of SP and antidepressant response is unclear. Both serotonin as well as norepinephrine re-uptake inhibitors reduced SP levels.

Stress is thought to play a role in the etiology of depression and contribute to the episodic relapses in individuals suffering from depression [73]. A variety of preclinical stress paradigms have been developed such as: immobilization, social deprivation and maternal deprivation. Following immobilization stress c-fos positive neurons were observed in the central nucleus of the amygdala suggesting activation of these neurons by stress [74]. Interestingly, many of these neurons were surrounded by SP immunoreactive fibers, suggesting SP input (directly or indirectly) onto these neurons. This is consistent with SP containing projections from the medial to the central nucleus and the presence of NK_1 receptors on central nucleus neurons. Immobilization stress also leads to a decrease in the number of NK_1 receptors in the amygdala [75]. The decrease in receptor number may represent endocytosis of somatodendritic receptors as the NK_1 receptor has been shown to undergo internalization following agonist stimulation or release of endogenous stores of SP [48, 49] as discussed previously. Internalization of the NK_1 receptor in response to stress was directly tested in gerbils that express an NK_1 receptor with human-like pharmacology allowing one to examine the effect of nonpeptide NK_1 receptor antagonists. Smith *et al.*, (1999) [76] observed that immobilization stress (1 hr) increased NK_1 receptor internalization in the basolateral nucleus of the amygdala, interpreted as reflecting local release of SP. Pretreatment with an NK_1 receptor antagonist blocked the receptor internalization, however acute administration of imipramine was without effect. Nonetheless, chronic administration of antidepressants to rats has been shown to decrease levels of SP in the amygdala suggesting that reduction in SP neurotransmission may underlie the actions of both NK_1 receptor antagonists and established antidepressants.

When separated from their mothers and littermates, neonates emit vocalizations that are blocked by pretreatment with anxiolytics and antidepressants [77, 78]. Using maternal separation as the stressor, Kramer *et al.* (1998) [79] showed that internalization of NK_1 receptors in the basolateral amygdala of guinea pig pups was increased. The vocalizations in guinea pig pups coincided with receptor internalization and the vocalizations could be blocked by intra-

amygdala infusion of an NK_1 receptor antagonist [80]. Finally, in a preliminary study, NK_1 expressing cells in the basolateral nucleus were ablated by administration of a substance P-toxin conjugate, substance P-saporin. Cells expressing the NK_1 receptor selectively take up the toxin and are killed. Animals administered this toxin exhibited reduced anxiety as measured in the elevated plus maze (EPM) and in the open field test where they spent less time immobilized [81].

The evidence suggests that in addition to local SP containing interneurons modulating the activity of projection neurons, amygdalar efferents containing SP may also participate in anxiogenesis in other brain areas. For example, SP containing neurons project from amygdala to periaqueductal gray (PAG) [82] where infusion of SP agonists also elicit anxiety and aversion [83-86]. Glutamate containing neurons in the PAG are the likely targets of SP as a majority of cells labeled for the NK_1 receptor were also positive for glutamate [87].

In summary, the amygdala is rich in SP and NK_1 receptors that likely participate in the response to stress. SP is released in the amygdala by a variety of stressors and activates the NK_1 receptor localized to GABA containing interneurons. NK_1 receptors in the amygdala and associated projection areas participate in the expression of anxiety-like behaviors and their blockade as discussed later gives rise to behavior interpreted as reduced anxiety.

DORSAL RAPHE

Antidepressants are thought to elicit their therapeutic effect as a result of elevating levels of serotonin (5HT). Forebrain 5HT arises from cells in the dorsal and median raphe, while it is the 5HT containing cells of the medullary raphe that project to the spinal cord. In the rat, 5HT and SP are colocalized within neurons of the medullary raphe nuclei, however there is no evidence that SP serves as a co-transmitter in forebrain projecting median and dorsal raphe systems in the rat [88-90]. Nonetheless, a delicate network of SP immunoreactive fibers with particularly intense staining in the ventral portion of the DRN has been described [91]. Some of these fibers may originate from the habenula nuclei [92]. An electron microscopic and double labeling study in the DRN and adjacent periaqueductal gray area indicated 5HT labeled terminals make contact on SP containing soma and dendrites and in turn SP containing terminals appose GABA containing cells [93]. Immunolocalization of the NK_1 receptor to GABA positive cells in the DRN has been reported [94], however this is at odds with other reports discussed below suggesting localization to glutamatergic containing neurons.

General autoradiographic surveys of NK_1 receptor distribution in rat brain have reported moderate to high densities of binding sites in the dorsal raphe [25, 95, 96]. Similar results are obtained using antibodies against the NK_1 receptor [64, 97], which provide a more detailed anatomical resolution including double and triple labeling to identify the phenotype of the cell expressing the NK_1 receptor. NK_1 receptors are not present on serotonergic cells but are found in the neuropil surrounding 5HT labeled cells [87, 98, 99]. The NK_1 receptors are likely to be present on glutamatergic cells in the DRN [87] which is consistent with electrophysiological studies discussed below.

There is limited information on the distribution of SP and NK_1 receptors in the human DRN. In contrast to the rat, substance P and serotonin are co-localized in a significant number of dorsal raphe neurons of primates including man [100-102]. However, low levels of NK_1 receptor mRNA expression and binding sites are found in the human and monkey in contrast to the moderate to high density found in rat [69, 103]. It will be important to confirm in man the reported low level of NK_1 receptors and to identify their cellular localization at a level of anatomical detail approaching that available for the rat.

A number of studies with NK_1 receptor antagonists were carried out in the guinea pig due to the NK_1 receptor having a similar pharmacology in guinea pigs and humans [104, 105]. Systemic injection of an NK_1 receptor antagonist (L-760735) increased the firing of DRN neurons [103]. However, there was no effect on cell firing following direct application of L-760735 into the DRN consistent with a paucity of NK_1 receptors in the guinea pig DRN. Similarly, in a slice preparation from guinea pig brain, neither SP nor L-760735 affected the firing of DRN neurons. However, iontophoresis of L-760735 into the lateral habenula elicited a 2.5-fold increase in the firing rate of DRN cells suggesting that a habenula-DRN pathway may be responsible for driving the activity of DRN cells. The ability to detect increased DRN activity following iontophoretic application of an NK_1 receptor antagonist to the habenula implies either a widespread innervation of the raphe by individual cells of the habenula or a greater spread of the antagonist throughout the habenula than may normally follow iontophoresis. The habenula-DRN projection, at least in the rat, is likely to represent a monosynaptic GABAergic circuit [106] although there is also a polysynaptic pathway that is thought to be mediated in part by SP [92]. Thus, in the guinea pig, blockade of an excitatory SP input onto habenula neurons appears to release the DRN from inhibitory GABAergic input arising in the habenula.

In the rat, SP appears to influence raphe cell firing indirectly through glutamatergic interneurons. In a slice preparation, SP increased excitatory post synaptic currents in 5HT neurons that was blocked by administration of glutamate receptor antagonists [107]. This is consistent with the report that ~50% of NK_1 positive cells in the DRN are also positive for glutamate [87]. Yet, an *in vivo* study in the rat paints a very different picture [108]. Recording from DRN cells following infusion of SP, the majority of cells exhibited either pure inhibition (66%) or a transient excitation followed by a longer lasting inhibition (17%) with a minority of cells excited by SP. A glutamate antagonist blocked the inhibitory as well as the excitatory actions of SP. The SP-elicited inhibition was also prevented either by systemic or direct infusion of a $5HT_{1A}$ antagonist suggesting that the inhibition of DRN firing could be due to local release of 5HT. These conflicting results could result from sampling different populations of neurons or be influenced by the absence of an intact neuronal input in the slice preparation (e.g. noradrenergic input) or the use of anesthesia *in vivo*. While the effect of SP on DRN firing needs to

be resolved, there appears to be a consistent finding that SP mediates its effect in the DRN, at least in part, through glutamate.

An inhibitory effect of SP on DRN cell firing is consistent with reports that a reduction in NK_1 receptor function was associated with an increase in activity of DRN neurons. Santarelli *et al.* (2001) [109] reported an increase in the *in vivo* basal firing rate of DRN neurons in mice (129 SvEV strain) with genetic deletion of the NK_1 receptor compared to wild type controls (however see Froger *et al.* (2001) [99]). In wild type controls they observed a similar increase in the firing of DRN cells following systemic administration of an NK_1 receptor antagonist. Increased DRN activity was also obtained in guinea pigs after a single administration of NK_1 antagonist [103] or following 7 to 14 days of dosing to rats [110]. In general a 2-fold increase in the basal activity of DRN cells was observed in either knockout animals or animals treated with an NK_1 receptor antagonist. In contrast to guinea pigs, in which NK_1 receptor mediated changes in the activity of DRN neurons appears mediated through habenua-DRN afferents, it has been proposed that NK_1 receptor antagonists acting locally increase the firing of DRN cells in part through desensitization of $5HT_{1A}$ receptors located on 5HT containing neurons.

$5HT_{1A}$ receptors are somatodendritically localized to 5HT containing cells in the DRN where they provide auto feedback to control the activity of these neurons [111]. *In vivo*, the reduction in firing of 5HT cells following LSD (presumed to be mediated through the $5HT_{1A}$ receptors) was attenuated following subacute or chronic treatment of rats with the NK_1 receptor antagonist CP-96,345 [110]. It can be argued that LSD has a multitude of pharmacological effects, however a similar effect was observed in NK_1 receptor knockout mice given the selective $5HT_{1A}$ autoreceptor agonist 8-OH DPAT. Compared to wild type control animals the efficacy of $5HT_{1A}$ receptor agonists to reduce DRN cell firing was significantly attenuated, consistent with a loss of $5HT_{1A}$ receptor function in these NK_1 receptor knockout mice [99, 109]. Behaviorally, 8-OHDPAT-induced hypothermia was also diminished in the NK_1 receptor knockout mice[99]. However, it is important to note that administration of $5HT_{1A}$ receptor antagonists has been shown to be without effect or produce only a small increase in basal firing rates (reviewed in Pineyro and Blier [112]). This may be due in part to the effects of anesthetics and a suppression of basal firing rates as Fornal *et al.* (1996) [113] have shown that in freely moving cats the $5HT_{1A}$ receptor antagonist, WAY 100635 increased cell firing by ~65% depending on the behavioral state of the animal. However, an increase in firing rate following treatment with either NK_1 receptor antagonists or in NK_1 receptor knockout mice has been observed under conditions of anesthesia.

Commons and Valentino [87] proposed that NK_1 receptors on glutamatergic cells excite a subpopulation of cells in the dorsomedial DRN that in turn inhibit other cells through the release of 5HT, acting on $5HT_{1A}$ autoreceptors. If NK_1 receptors on glutamate neurons are genetically deleted or pharmacologically blocked, the "inhibitory" 5HT neurons are no longer activated and the majority of DRN cells would be released from inhibition consistent with what has been observed. Furthermore, the finding of Liu and Aghajanian [107], that activation of NK_1 receptors increased the firing of DRN cells through excitation of a local population of glutaminergic inputs could be reconciled with Commons and Valentino if one assumes Liu and Aghajanian were recording from a population of dorsomedial DRN cells.

If blockade of NK_1 receptors activates the DRN, then the failure to find an increase in 5HT neurotransmission in terminal areas of the DRN is surprising, although, to date only the frontal cortex and hippocampus have been examined [79, 99, 114, 115], (H. Rollema, Pfizer, Groton USA, unpublished). Whether the 2-fold increase in cell firing is insufficient to increase 5HT in frontal cortex and hippocampus or alternatively the increase is occurring in other areas remains to be determined. Small changes in discrete areas similar to the modest change in 5HT turnover observed in mice lacking $5HT_{1A}$ receptors would be expected, particularly if desensitization of the $5HT_{1A}$ receptor plays a role [116]. The amygdala may represent an interesting terminal area to look for changes in 5HT given the localization of NK_1 receptors in the dorsomedial nucleus of the DRN which in turn projects to the amygdala [117]. Confirmation of the proposed regio-selective excitation and inhibition of DRN cell firing is needed.

The excitation of the DRN has been suggested to contribute to the antidepressant effects of the NK_1 receptor antagonists. However, the failure to find changes in 5HT release in terminal areas coupled with the lack of sexual side effects observed in the clinic suggest a subtle effect on the 5HT system. Furthermore, given the different localization of NK_1 receptors in primates and rats, extrapolating the location of NK_1 receptors in rodent DRN to that of man may be questionable.

LOCUS COERULEUS

Since the discovery of the tricyclic antidepressants that selectively block the reuptake of norepinephrine (NE), considerable attention has focused on the role of NE in depression [118-120]. The locus coeruleus (LC) contains a majority of NE containing cells that send a diffuse projection throughout the brain. The frontal cortex, amygdala, hippocampus and the dorsal raphe all receive NE input.

Early studies using radioimmunoassays reported moderate to high levels of SPLI in the human LC ([65] and reviewed therein). Using immunohistochemistry several laboratories have reported a few scattered fibers positive for SP in the human LC [121, 122], although a relatively dense network of fibers has also been reported [123]. The LC contains a high level of NK_1 receptor mRNA expression consistent with the moderate to dense labeling of binding sites determined by quantitative autoradiography [19, 69, 124]. The high level of message and binding sites in man suggests that many of the NK_1 receptors are localized to cells intrinsic to the LC. As discussed below for the rat, it would be interesting to know if the NK_1 receptor is expressed on NE containing cells in man.

In the rat, SPLI is localized to terminals forming axondendritic contacts with TH positive cells [125, 126]. Autoradiographic studies, using a variety of ligands have

described dense binding to NK_1 receptors in rat LC [25, 127-130]. Immunohistochemical studies confirm a significant density of NK_1 receptors in the LC [97] and double labelling studies localize the NK_1 receptor to noradrenergic containing neurons with the majority of NE containing cells positive for NK_1 receptors [94, 131, 132].

The majority of cells in the rat LC are excited by iontophoretic application of SP with a time course that is slow in onset and exhibits no tachyphylaxis [133, 134]. Similar SP-induced excitation of LC neurons is observed in slice preparations from rat [135] and guinea pigs [136, 137] as well as following local infusion into the LC of guinea pigs [138]. While SP is excitatory, the effect of NK_1 receptor antagonists on basal firing of LC neurons is controversial. *In vivo*, antagonists have been shown to be without effect on basal firing-rates in rats [139] and guinea pigs [128, 137, 138, 140] although Millan *et al.* (2001) reported a 50% increase in firing rates following administration of GR205171 [114]. Chronic administration of an NK_1 antagonist, L-730735 did not alter the firing rate of LC neurons, however in guinea pigs it induced an increase in burst firing similar to that observed following chronic imipramine [140]. In contrast to imipramine, L-730735 did not desensitize somatically located α2 receptors suggesting that it was influencing the burst activity of LC neurons through a different mechanism. The firing rate of LC cells in animals with a genetic disruption of the NK_1 receptor has not been described, but may help to elucidate how NK_1 receptors influence the activity of LC cells.

The increased activation of LC neurons following local application of SP is associated with elevated levels of NE in terminal areas [137, 138]. An NK_1 receptor antagonist (GR205171) has been reported to elicit a dose dependent elevation in dialysate levels of NE in rat frontal cortex consistent with the reported increase in LC firing in this study [114]. However, in mice GR205171 is without effect on NE levels in frontal cortex [115]. Exactly how agonists and antagonists of the NK_1 receptor can increase NE turnover and cell firing is unclear. Understanding the conditions under which NK_1 receptors are activated and in turn stimulate the activity of LC neurons will be important given that NK_1 receptors are similarly localized to NE containing neurons of the LC in both man and preclinical species and that stress-induced activation of the LC may be blocked by administration of NK_1 receptor antagonists [132].

SUBSTANCE P, NK_1 RECEPTORS AND "EMOTIONAL BEHAVIOR"

In general, infusion of substance P and SP fragments are reported to elicit anxiogenic effects in a number of behavioral assays. Substance P elicits an anxiogenic effect when infused into the ventricles [141], that is proposed to result from activation of NK_1 receptors in the lateral septum [142, 143]. Microinjection of SP into the dorsal periaqueductal gray also increases anxiety as measured in the elevated plus maze [85]. The anxiogenic activity of SP can be attenuated by low doses of nitric oxide inhibitors suggesting that nitric oxide plays some role [144]. Interestingly the N-terminal fragment SP 1-7, which has little affinity for the NK_1 receptor, elicits an anxiolytic effect when infused into the PAG [145, 146] and in the marmoset predator model of fear/anxiety [147]. Furthermore, an NK_1 receptor antagonist does not block the anxiolytic effect of SP1-7. Thus, the anxiolytic effects observed for SP may in some cases result from generation of the N-terminal fragments of SP. However, SP has been reported to have anxiolytic effects when given systemically [148] or when infused into the ventral pallidum, an effect that is attenuated by pretreatment by the NK_1 receptor antagonist WIN 51708 [149, 150] suggesting SP-induced anxiolysis in the ventral pallidum is mediated through NK_1 receptors. Despite these findings, SP has been shown to be anxiogenic in many brain areas and in a variety of behavioral models consistent with the proposal that antagonists of the receptor are anxiolytic. This is further buttressed by a number of studies demonstrating anxiolytic and antidepressant activity of NK_1 receptor antagonists in preclinical behavioral models.

PRECLINICAL ACTIVITY IN MODELS OF ANXIETY AND DEPRESSION

There are innumerable experimental models of anxiety and depression that are used preclinically. The face validity, predictive validity as well as the "emotional" state the various behavioral paradigms purport to model is beyond the scope of this review. However, the wide range of assays in which NK_1 receptor antagonists and NK_1 receptor knockout mice have shown "anxiolytic" and "antidepressant" activity (Table 2) argues strongly for a role of SP in anxiety and depression and is supported by the currently available clinical data. In assessing the effects of NK_1 receptor antagonists in the various models, species differences in the pharmacology of the antagonists needs to be considered [104, 105] given that many of the compounds have reduced affinity for the rodent NK_1 receptor and thus often require high doses to observe an effect. Interpretation of these effects is strengthened by the use of "inactive" enantiomers that exhibit negligible affinity for the NK_1 receptor.

Preclinically the "emotion" of fear and anxiety are operationally defined physiologically as changes in blood pressure, tachycardia, excessive glucocorticoid secretion or behaviorally as avoidance, freezing or escape [151, 152]. The elevated plus-maze (EPM) is often used to test drugs for their anxiety reducing properties and has been used extensively to phenotype genetically modified animals. The EPM takes advantage of unconditioned or spontaneous preference for enclosed alleys and avoidance of open alleys with the assumption that open arms evoke a stronger fear/anxiety reaction than staying in the enclosed arms. With respect to NK_1 receptor antagonists, the evidence for their anxiolytic activity in the EPM is equivocal. Thus, structurally diverse compounds, RP67580, MK-869, L-742694, CP-122,721 and CP-99,994 (Fig. 2) were active in either mice or gerbils in the EPM at doses that did not effect spontaneous locomotor activity [109, 153, 154]. Furthermore, the anxiolytic activity for RP67580 is reported to be enantioselective [109]. In contrast, when tested in rats and guinea pigs, GR205171 and L-760735 lacked anxiolytic activity in the EPM [155]. The data derived from mice with genetic deletion of the NK_1 receptor are also mixed with one group reporting an anxiolytic phenotype in the EPM [109] while

Table 2. Effects of SP and NK_1 Receptor Antagonist in Behavioral Models of Depression and Anxiety

Behavioral Test/Species	Compound	Outcome	Reference
Elevated Plus Maze/Mouse	FK888	Anxiolytic	[141]
" "	SP/SPmethyl ester	Anxiogenic	ibid
" "	RP67580	Anxiolytic	[109]
" "	NK_1 receptor knockout mouse	Anxiolytic	ibid.
" "	NK_1 receptor knockout mouse	No Effect	[168]
" "	NK_1 receptor knockout mouse	No Effect (trend)	[156]
" "	SP (blocked by WIN51708)	Anxiogenic	[140]
Elevated Plus Maze/Rat	GR205171	No Effect	[156]
Elevated Plus Maze/guinea pig	L-760735	No Effect	[156]
Elevated Plus Maze/gerbil	MK-869 L-742694 CP-122721 CP-99,994	anxiolytic	[200]
Social Interaction/rat	NKP608	Anxiolytic	[159]
" "	CGP 49823	Anxiolytic	[161]
" "	NKP608	Anxiolytic	[157]
Social Interaction /gerbil	NKP608	Anxiolytic	[158]
" "	L-760735	Anxiolytic	[160]
Social Interaction/mouse	TAC1 knockout	Anxiolytic	[155]
Open Field/mouse	TAC1 knockout	Anxiolytic	[155]
Vogel Conflict Test/rat	GR205171	Anxiolytic	[152]
Thatcher/Britton Conflict Test/mouse	TAC1 knockout	Anxiolytic	[155]
Separation-Induced vocalization/ mouse	RP67580	Anxiolytic/Antidepressant	[109]
" "	NK_1 receptor knockout mouse	Anxiolytic/Antidepressant	ibid
" "	GR205171	Anxiolytic/Antidepressant	[163]
" "	NK_1 receptor knockout mouse	Anxiolytic/Antidepressant	ibid
Separation-Induced vocalization/ rat	GR205171	No enantioselectivity	[201]
Separation-Induced vocalization / guinea pig	L-733060 L-760735	Anxiolytic/Antidepressant	[79]
" "	SSR240600	Anxiolytic/Antidepressant	[137]
" "	L-733060 GR205171 LY303870 (partial inhibition) CGP49823 (partial inhibition)	Anxiolytic/Antidepressant	[163]
Forced Swim Test/mouse	TAC1(preprotachykinin gene) knockout	Antidepressant	[155]
" "	NK_1 receptor knockout mouse	Antidepressant	[156]

(Table 2) contd....

Behavioral Test/Species	Compound	Outcome	Reference
" "	GR205171(no enantioselectivity) L-760735	Antidepressant	ibid
" "	NK_1 receptor knockout mouse	Antidepressant	[96]
Tail Suspension/Gerbil	L-760735	Antidepressant	[156]
" "	MK-869 L-742694 L-733060 CP-99, 994 CP-122721	Antidepressant	[169]
Tail Suspension/Mouse	NK_1 receptor knockout mouse	Antidepressant	[156]
" "	GR205171	No Effect	ibid
" "	Preprotachykinin knockout	Antidepressant	[167]

mthers failed to observe reduced anxiety in the NK_1 receptor knockout mice relative to wild type controls [155, 156]. These results could represent differences in the background of the mouse strains used to generate the knockout phenotype. However, genetic deletion of the gene encoding for SP/NKA yields a mouse with an anxiolytic phenotype as measured in the zero maze, a methodological variant of the EPM.

In contrast to the equivocal results obtained in the EPM, NK_1 receptor antagonists consistently exhibit anxiolytic activity in the social interaction test. As with the EPM the social interaction test takes advantage of an animal's natural tendency to reduce social contact or enter into highly lit areas as a function of an animal's state of anxiety and the perceived averativeness of the test environment. In rats, mice and gerbils acute administration of NK_1 receptor antagonists increased the duration of social contact in a manner similar to that observed with benzodiazepines [157-160]. Interestingly, animals treated chronically with CGP49823 did not show any rebound anxiety upon withdrawal in contrast to the increased anxiety e.g. reduced social interation, following withdrawal from diazepam [161]. In fact, rebound withdrawal from 28 days of treatment with diazepam was blocked by central administration of FK888 suggesting an interaction of the tachykinin and GABAergic systems [162]. Although able to attenuate the rebound anxiety, the NK_1 system is not required for the anxiolytic activity of benzodiazepines since diazepam was still able to increase entry into the open arms of the EPM in NK_1 receptor knockout animals [109].

Separation of neonates from their mothers and littermates elicits distress vocalizations (both audible and inaudible) and a concomitant increase in plasma levels of ACTH and cortisol [77, 78]. Anxiolytic compounds, 5HT and NE uptake inhibitors and recently NK_1 receptor antagonists have demonstrated activity in the separation-induced vocalization paradigm.

In guinea pigs that express an NK_1 receptor with a pharmacology similar to that of man, a variety of structurally diverse NK_1 receptor antagonists blocked separation-induced vocalization and exhibited behavioral effects similar to diazepam and buspirone and the antidepressants fluoxetine and venlafaxine [79, 137, 163]. A similar anxiolytic/antidepressant phenotype was observed in the NK_1 receptor knockout mouse [109, 155]. Similar to the results obtained in the EPM, diazepam reduced separation-induced vocalization in NK_1 knockout mice [109]. Although diazepam blocked separation-induced vocalization in knockout and wild type mice as well as in guinea pigs, it did not affect vocalizations induced by central infusion of a SP agonist in guinea pigs [164]. This suggests that the NK_1 and benzodiazepine/GABA systems act to reduce anxiety through distinct mechanisms, however it may be through activation of similar pathways. For example, as with the benzodiazepines, NK_1 receptor antagonists may be acting through the amygdala to reduce anxiety.

The amygdala has long been associated with conditioned fear [52] and may play a role in vocalizations elicited by maternal deprivation [165]. Kramer *et al.* (1998) [79] demonstrated that separation of guinea pig pups from their mothers for 5 minutes increased NK_1 receptor internalization in the basolateral nucleus of the amygdala suggesting an increase in SP release. This finding has been replicated [137] and the results further elaborated. Analysis of the time course of the vocalizations and receptor internalization indicated that the time at which the greatest number of cells exhibited receptor internalization corresponded to the peak in vocalizations. Infusion of an NK_1 receptor antagonist into the basolateral amygdala blocked separation-induced vocalization in guinea pig pups further strengthening the role of amygdalar SP neurotransmission in eliciting distress vocalization [80]. However, imipramine which attenuates vocalizations in guinea pig and rat pups had no effect on internalization of NK_1 receptors in the gerbil [76]. It would be interesting to demonstrate whether, in the same species imipramine was able to reduce vocalization independent of a reduction in NK_1 receptor internalization (i.e. changes in SP neurotransmission).

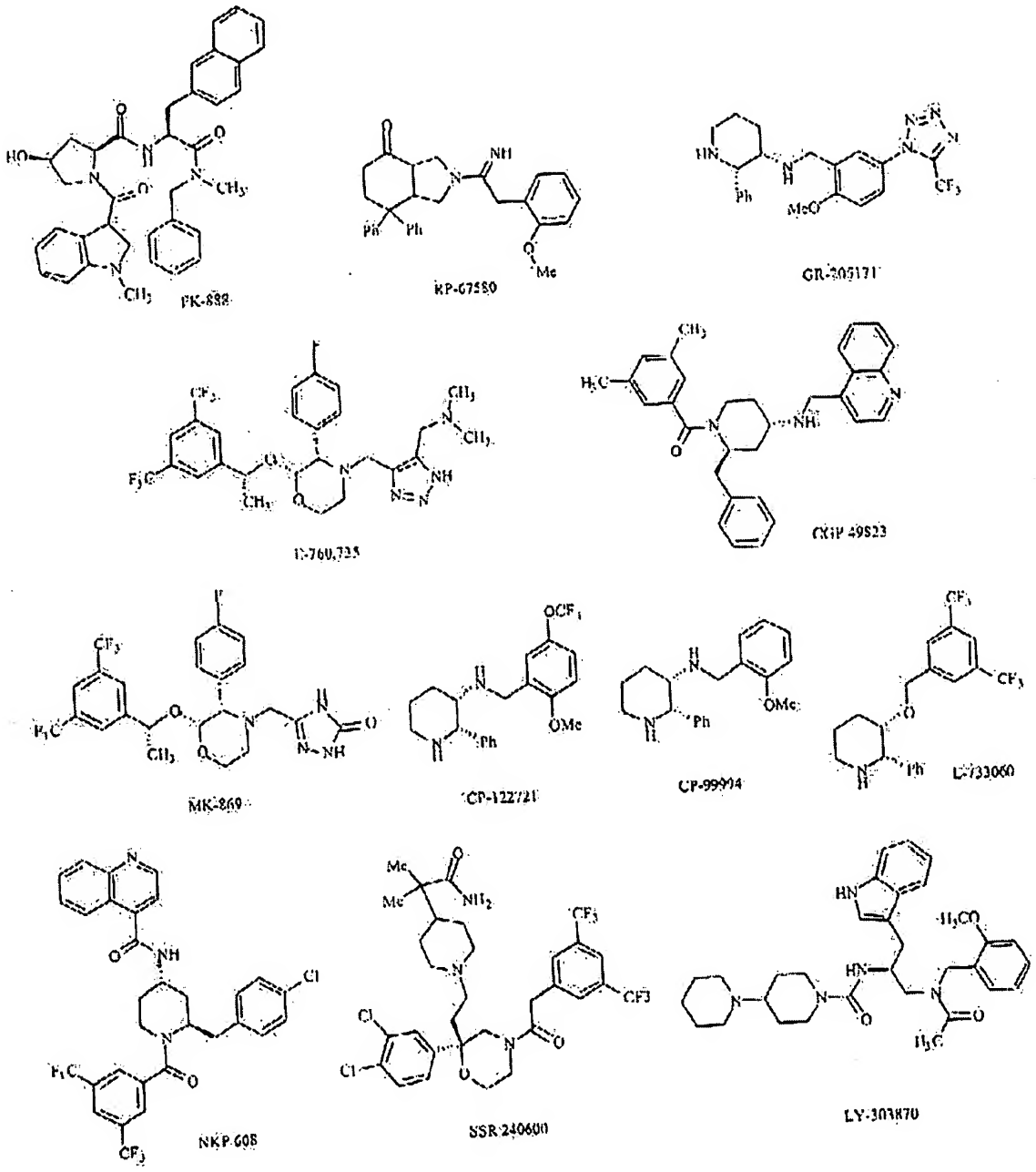

Fig. (2). Chemical Structure of NK_1 Receptor Antagonists.

The forced swim (FST), tail suspension assays and olfactory bulbectomy represent models used for detecting antidepressant-like activity of novel compounds [166]. The FST and tail suspension assays have the advantage of detecting compounds after a single administration, but are at odds with the need to administer antidepressants chronically to obtain a clinical benefit. In contrast, the olfactory bulbectomy model and chronic mild stress paradigms require chronic administration of compounds thus mimicking clinical use.

Mice lacking either the NK_1 receptor or the gene encoding for SP/NKA exhibited a decrease in immobility time in the FST similar to that observed with SSRIs and TCAs suggesting antidepressant potential [109, 160, 167, 168]. GR205171 was also active in the mouse FST although with marginal enantioselectivity [168]. Mixed results were obtained in the tail suspension paradigm. The phenotype observed after deletion of the NK_1 receptor or SP was consistent with antidepressant activity, however both GR205171 and L-760735 were inactive in the mouse and gerbil tail suspension assays, respectively [155, 167]. In contrast, antidepressant activity was obtained with a variety of other NK_1 receptor antagonists in the gerbil tail suspension assay [169]. Although these investigators tested neither GR205171 nor L-760735, close analogs of L-760735 were active (i.e. MK-869 and L-733060). Unfortunately, the enantioselectivity of the drug effect was not evaluated in these papers.

The olfactory bulbectomy model is another antidepressant model in which removal of the olfactory bulb in mice and rats elicits an increase in open field activity that is reversed by chronic antidepressant treatment. Olfactory bulbectomized mice with a deletion of the gene encoding for SP/NKA did not increase their open field activity relative to sham operated animals and were significantly less active than their wild type littermates that had also been bulbectomized [167]. It would be interesting to know if infusion of SP restored the depressed phenotype.

Chronic mild stress leads to changes in the biochemistry and behavior of animals. Behaviorally the animals exhibit a reduced response to rewarding stimuli as measured by diminished consumption of sucrose. This reduced consumption can be reversed by chronic treatment with antidepressants or electroconvulsive shock, but not by benzodiazepines [170, 171]. Five weeks of treatment with the NK_1 receptor antagonist NKP608 reversed the stress-induced decrease in sucrose consumption without affecting sucrose intake in non-stressed animals [172]. Given the low doses of NKP608 that were active (0.03 and 0.1 mg/kg) and the inverted dose response curve, it would be interesting to replicate these findings as well as those observed in the olfactory bulbectomy model with other NK_1 receptor antagonists.

Placing animals into stressful situations is the major feature of many of the preclinical models of depression. A number of investigators have demonstrated that 1) stress is a significant contributor to the development of depression [73], 2) there are significant reductions in hippocampal volume in depressed patients [22], 3) stress has a significant effect on neurogenesis in the adult hippocampus of rodents [173] and 4) chronic treatment with antidepressants block the effect of stress and increase neurogenesis when given alone [174]. For example, following maternal separation rat pups exhibit a reduction in neurogenesis with a corresponding decrease in hippocampal volume that can be prevented by chronic treatment with fluoxetine [175]. Male subordinate tree shrews subjected to chronic psychosocial stress by daily exposure to dominant animals for 35 days, exhibited decreased cell proliferation and hippocampal volume [176]. However, administration of L-760735 or clomipramine concomitantly with the stress for 28 days attenuated the stress-induced decrease in cell production and reduction in hippocampal volume [177]. In a preliminary investigation, neurogenesis was increased in the hippocampus of mice with a genetic deletion of the NK_1 receptor [178]. Although antidepressants increased neurogenesis in wild type animals, no further increase was observed in the knockout mice. Since the knockout mice already had elevated levels of neurogenesis, it is unclear whether this represents a ceiling effect or that both citalopram and desipramine were acting through a common mechanism, the NK_1 receptor.

Taken together, the behavioral data support an antidepressant profile of NK_1 receptor antagonists and although mixed, the results suggest that the NK_1 receptor antagonists may also have anxiolytic activity.

CLINICAL ACTIVITY

Few experiments have been performed to examine the effect of stress on levels of SP in man. Blood samples obtained from 47 inexperienced tandem-parachutists 2 h before, immediately after, and 1 h after a parachute jump were analyzed for plasma concentrations of substance P. Substance P concentrations were effected by the jump stress, however, subjects higher in anxiety at the point of jumping (exit) displayed higher substance P values at all three time points compared to the "low-anxiety" jumpers [179].

In a second study, 22 male volunteers were subjected to a battery of psychological tests at the height of the Iraqi Scud missile attacks on Israeli cities during the 1991 Persian Gulf War and again after the cessation of hostilities. Venous blood samples were taken at each time point. Psychological testing indicated levels of anxiety were higher during the war than they were after the war ended, and both anxiety and anger during the hostilities were significantly elevated in comparison with prewar data. During the war plasma levels of substance P were significantly elevated [180].

Although limited, the data suggest that SP is elevated in man as a consequence of stress.

SP LEVELS IN DEPRESSION

Rimon *et al.* (1984) [181] first reported an increase in SPLI in the CSF of patients with major depression compared to schizophrenics and normal controls. They noted however, that the SPLI material was likely to be C-terminal fragments of SP. In addition, they reported elevated levels of the N-terminal fragment SP (1-7) in the CSF of depressed patients. This study was followed by another study in schizophrenic patients, but had 3 patients with depression as part of a psychiatric control group. The mean CSF SP concentration of patients with major depression was higher than the corresponding mean concentration of the other patients in the nonschizophrenic group [182]. SPLI levels appeared similar in a study of acutely manic, euthymic, or depressed unmedicated unipolar and bipolar subjects in addition to a second outpatient group of euthymic bipolar patients (on and off lithium) and normal volunteers [183]. In this study it was noted that SPLI levels increased after a course of freeze thawing that raised methodological concerns. A later study raised further methodological issues reporting that SP (1-11) could not be detected in CSF samples, but that N-terminal

fragments of SP extended at the N-terminus or preprotachykinins are present in CSF [184, 185].

Martesson *et al.* (1989) [186] in an open study of depressed inpatients (n=9 completers), looked at the effects of fluoxetine on N-terminally extended SP in CSF. The mean level of N-terminally extended SP was unaffected by fluoxetine treatment. With methodological improvements for detecting SP and its fragments [187] (Table 3), it will be interesting to further explore SP concentrations in CSF of depressed patients to clarify the levels of these various peptides. It is of interest given that SP (1-11) or SP (7-11) that interact with the NK_1 receptor are anxiogenic in preclinical studies while the N-terminal fragments such as SP (1-7) are often found to be anxiolytic. Thus, determining the relative proportion of parent and fragments might yield some clues to the role of SP in depression. Resolution of the conflicting data might provide an opportunity for using CSF levels of SP as a biomarker for depression. Clearly elevated CSF levels of SP are not limited to depressed individuals as similar increases are observed in patients with chronic pain syndromes [188]. Nonetheless, depression-like symptoms are often found in patients suffering from chronic pain.

In contrast to the previous studies measuring SP levels in CSF, Bondy *et al.* (2003) measured plasma levels of SP in patients with major depression [189]. However, plasma and cerebrospinal fluid levels of SP are reported to be positively correlated [190]. Twenty three patients, drug free at least 1 week prior to plasma sampling, were compared to 33 normal controls. At baseline the drug free depressed patients had significantly higher plasma levels of SP than controls. Following 4 weeks of treatment with mirtazepine (n=15) paroxetine (n=4) or nonselective antidepressants (n=4) patients with a decrease in SP responded better to treatment than those with increased plasma SP levels.

Infusion of SP (3 pmol/kg/min) into 12 healthy subjects significantly increased cortisol levels in plasma and worsened subjects' mood [191]. Although consistent with a role of SP in depression, the central effects of peripherally administered SP needs to be further explored.

If SP is elevated in depression and receptor internalization occurs in man in response to increased neurotransmission one might expect to observe decreased binding to NK_1 receptors in postmortem tissue from depressed patients relative to controls. Burnet *et al.* (2000) [192] looked at NK_1 receptor density in the cingulate cortex of subjects with unipolar (n=14), or bipolar (n 13) depression. Using quantitative autoradiography, there was no difference in the binding of [^{125}I]-BH-SP between the depressed patients and controls. If the density of NK_1 receptors in superficial layers was compared to deeper laminae, this ratio was reduced in subjects with unipolar depression. Expressing the binding as a ratio may reduce the intersubject variability in NK_1 receptor expression, nonetheless, the lack of a significant overall decrease in receptor levels in cingulate cortex cannot be dismissed. In a second study, the binding of [^{125}I]-BH-SP was examined in the rostral subfrontal cortex (Brodmann area 47) of 12 depressed subjects. The average binding of [^{125}I]-BH-SP was decreased across all cortical laminae [193]. The differences between these two studies are that the later study had a shorter postmortem interval and samples were obtained from a different cortical area and limited to the left as opposed to both hemispheres.

While changes in SP and receptor number are consistent with a role in depression, care must be taken not to over interpret the results. Single concentration estimates of receptor density as carried out in these studies falls prey to the difficulty of deciphering changes in receptor affinity from changes in receptor number. Further studies are warranted and may benefit from an evaluation of mRNA levels encoding the receptor in addition to binding density. NK_1 receptors are rapidly internalized, subjected to recycling to the cell surface or lysosomal degradation making interpretation of changes in receptor number difficult while peptide levels reflect a dynamic interplay of synthesis, release and degradation. Thus, these static measurements are unlikely to accurately predict synaptic concentrations of SP or activation of the NK_1 receptor.

CLINICAL STUDIES WITH NK_1 RECEPTOR ANTAGONISTS

Several studies have been carried out in depressed patients to evaluate the antidepressant activity of selective NK_1 receptor antagonists (Table 4). Scientists from Merck first reported on a multicenter, double blind study in 213 depressed subjects randomly assigned to placebo, paroxetine

Table 3. Relative Binding Activity of SP and Fragments

Peptide	Relative Binding at NK_1 Receptor
Substance P (H-Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2)	100
$SP_{(1-4)}$	<0.1
$SP_{(1-7)}$	inactive
$SP_{(5-9)}$	<<1
$SP_{(4-11)}$	25
$SP_{(5-11)}$	3
$SP_{(7-11)}$	<<1

References [202-204]

(20 mg) or MK-869 (300mg). Efficacy measures using the Hamilton depression and anxiety scores and Clinical Global Impressions severity scale (CGIS) were taken at weeks 1, 2, 4 and 6 or at termination of the study. At week six, patients taking MK-869 had a mean decrease of 4.3 points on the HAM-D relative to baseline compared to a decrease of 3.6 points obtained with paroxetine [79]. Fifty four percent of patients receiving MK-869, 46% receiving paroxetine, and 28% in the placebo group had a significant response defined as a decrease of > 50% from baseline HAM-D score. Complete "remission" of symptoms occurred in 43% of the patients treated with MK-869 compared to 33 and 17% of patients on paroxetine or placebo, respectively. There were two other interesting observations 1) an anxiolytic effect was observed with MK-869 in this population of depressed subjects and 2) the incidence of sexual dysfunction in patients on MK-869 was considerably better than with paroxetine, 3% versus 26%, respectively.

A second study [16] conducted at 19 sites with 4 dose levels (10, 30, 100 and 300 mg) failed, with both MK-869 and fluoxetine not different from placebo control. Post hoc analysis stratifying subjects who scored > 26 or < 26 at baseline on the HAM-D revealed a dose dependent decrease in depressive symptoms in the subjects with greater depression (>26) suggesting that patients with more severe depression might be more likely to benefit from NK_1 receptor antagonists.

Kramer *et al.* (2004) reported that blockade of NK_1 receptors with L-759274 resulted in a significant reduction in HAM-D scores at the end of six weeks with a 37% responder rate relative to placebo (25%) and a significant reduction in anxiety as measured by the HAM-A in a group of depressed subject with a mean HAM-D score of ~ 28 with features of melancholic depression ([194] and cited in[195]). There was no active comparator in this study. In a presentation at the Association of European Psychiatry (2002), Kramer reviewed an 8 week trial with 91 patient/arm and 2 dose levels of L-759274 plus paroxetine and placebo controls. Unfortunately, both the response to L-759274 and paroxetine failed to distinguish from placebo control, which had an 8 point drop in HAM-D scores [196]. Finally, a large Phase III trial using MK-869 was recently reported in the lay press. While details were not available, MK-869 did not evidence a significant reduction in HAM-D scores in contrast to the active comparator which was significantly different from placebo.

Chappell recently revealed the results of a phase II trial in depressed patients using CP-122, 721 [197]. CP-122, 721 (10 and 30 mg BID) was tested in a 6 week, double blind, placebo controlled trial with fluoxetine (20 mg) as the comparator. The subjects were medication free, healthy outpatients with a primary diagnosis of MDD with a minimum total score of 22 on the 17-item HAM-D. Subjects were evaluated at 1, 2, 4 and 6 weeks using the HAM-D and CGI severity index. At week 6 subjects receiving placebo had on average a 7.5 point drop in HAM-D scores while those on 10 mg BID of CP-122, 721 benefited with a 9.5 point drop. HAM-D scores of patients on 30 mg BID of CP-1227, 721 decreased by 12 points, similar to the 13 point decrease in HAM-D scores observed in subjects receiving fluoxetine. Furthermore, like MK-869 there is accumulating evidence with CP-122721 for reduced sexual side effects based on standardized rating scales (ASEX) and sexual adverse event reports.

Glaxo has recently disclosed (company web site) that GW597599 was active against CO_2-induced panic attack with efficacy similar to alprazolam as measured using a visual analog scale. This represents the first demonstration of efficacy of NK_1 receptor antagonists against panic disorder, further extending their utility.

Table 4. Clinical Trials with NK_1 Receptor Antagonists

Compound	Dose tested	Sites	Duration	Comparator	Outcome
MK-869	300 mg, QD	multi	6 weeks	Placebo, Paroxetine	Equal to Paroxetine
MK-869	10 mg, QD 30 mg, QD 100 mg, QD 300 mg, QD	19 sites	6 weeks	Placebo Fluoxetine	Failed trial
L-759274	40 mg, QD	9 sites	6 weeks	Placebo	Significant effect
L-759274	2 doses	10 sites	8 weeks	Placebo Paroxetine	Failed trial
MK-869 (Phase III)		multi			Negative trial
CP-122, 721	10, BID 30, BID	Multi	6 weeks	Fluoxetine	Equal to Fluoxetine
GW-597599 (CO_2)-induced anxiety	15 mg, (QD)	-	Acute	Alprazolam	Similar efficacy to comparator on VAS

Finally imaging of NK_1 receptors in human brain have been conducted using an ^{18}F radiotracer with highest accumulation of the tracer occurring in brain areas rich in NK_1 receptors such as the caudate, putamen and amygdala [198]. Normal healthy subjects were treated for 14 days with MK-869 and then scanned at plasma trough levels of drug. Both 300 and 125 mg doses of MK-869 produced greater than 90% blockade of the NK_1 receptor, while 10 mg was associated with approximately 60% occupancy. Although 300 mg of MK-869 was active in depression and associated with at least 90% receptor occupancy, there is no data available on the efficacy of lower doses of MK-869 in depression due to failed trials in the dose ranging studies. Thus, a fertile area of research will be to determine the level of receptor occupancy associated with efficacy in depression.

In summary, clinical studies with three different compounds demonstrate antidepressant efficacy in both mildly depressed as well as melancholic patients. Furthermore, the favorable side effect profile of the agents suggests a viable therapy particularly for people experiencing significant sexual side effects with currently available antidepressants. This has to be balanced against a number of trials in which NK_1 receptor antagonists failed to show activity. In addition to the previously mentioned negative trials, NKP608 another NK_1 receptor antagonist was reported on the Novartis web site to have been terminated from further development for depression although it is unclear whether this was due to side effects or lack of efficacy. To date there are three positive trials in depression, one positive trial in panic, several failed trials and at least 2 negative studies. To put these results in context, approximately half the antidepressant clinical trials with SSRIs fail to differentiate from placebo [199] similar to the success rate observed with NK_1 receptor antagonists.

SUMMARY

SP and the NK_1 receptor are localized to brain areas implicated in the pathophysiology of depression. The NK_1 receptors are often localized to interneurons in areas such as the frontal cortex, amygdala and dorsal raphe nuclei where they are able to influence output pathways or in the case of the locus coeruleus directly on the projection neurons. SP is released in response to stress as measured by internalization of the NK_1 receptor and blockade of the receptor has been shown to mitigate the effect of stress. Preclinical studies with structurally distinct antagonists have supported their potential for the treatment of depression and anxiety that has been confirmed in clinical trials. Their efficacy in depression is similar to the SSRIs, yet lacking in the sexual side effects associated with SSRIs. A trial with MK-869 suggests that NK_1 antagonists may have particular utility in the treatment of seriously depressed patients, however this requires further testing. Similarly, the recent disclosure that GW597599 is active against CO_2-induced panic is promising but needs confirmation in a Phase II outpatient trial with patients suffering from panic attacks.

ACKNOWLEDGEMENT

I would like to thank my colleagues Chris Schmidt and Jeff Sprouse for their critical reading of the manuscript.

REFERENCES

References 205-207 are related articles recently published in Current Pharmaceutical Design.

[1] Chang MM, Leeman SE. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. J Biol Chem 1970; 245 (18): 4784-90.

[2] Krause JE, Chirgwin JM, Carter MS, Xu ZS, Hershey AD. Three rat preprotachykinin mRNAS encode the neuropeptides substance P and neurokinin A. Proc Nat Acad Sci USA 1987; 84 (3): 881-5.

[3] Regoli D, Boudon A, Fauchere JL. Receptors and antagonists for substance P and related peptides. Pharmacol Rev 1994; 46 (4): 551-99.

[4] Otsuka M, Yoshioka K. Neurotransmitter functions of mammalian tachykinins. Physiol Rev 1993; 73 (2): 229-308.

[5] Steinberg R, Marco N, Voltsinis D, Derraid M, Rodier D, Souilliac J, *et al.* Expression and presence of septal neurokinin 3 receptors controlling hippocampal acetylcholine release during sensory stimulation in rat. Eur J Neurosci 1998; 10 (7): 2337-45.

[6] Hagan RM, Beresford IJ, Stables J, Dupere J, Stubbs CM, Elliott PJ, *et al.* Characterisation, CNS distribution and function of NK_3 receptors studied using potent NK_3 receptor antagonists. Reg Peptides 1993; 46 (1-2): 9-19.

[7] Stratton SC, Beresford IJ, Harvey PJ, Turpin MP, Hagan RM, Tyers MB. Anxiolytic activity of tachykinin NK_3 receptor antagonists in the mouse light-dark box. Eur J Pharmacol 1993; 250 (3): R11-2.

[8] Steinberg R, Alonso R, Oriebel G, Ben L, Jung M, Oury-Donat F, *et al.* Selective blockade of neurokinin-3 receptors produces antidepressant-like effects associated with reduced corticotropin-releasing factor function. J Pharmacol Exp Ther 2001; 299 (2): 449-58.

[9] Mileusnic D, Lee JM, Magnuson DJ, Hejna MJ, Krause JE, Lorens JB, *et al.* Neurokinin-3 receptor distribution in rat and human brain: an immunohistochemical study. Neuroscience 1999; 89 (4): 1269-90.

[10] Dam TV, Escher E, Quirion R. Visualization of neurokinin-3 receptor sites in rat brain using the highly selective ligand [^{3}H]senktide. Brain Res 1990; 506 (1): 175-9.

[11] Ding Y-Q, Shigemoto R, Takada M, Ohishi H, Nakanishi S, Mizuno N. Localization of the neuromedin K receptor (NK-3) in the central nervous system of the rat. J Comp Neurol 1996; 364 (2): 290-310.

[12] Emonds-Alt X, Proietto V, Steinberg R, Advenier C, Daoui S, Naline E, *et al.* Biochemical and pharmacological activities of SSR 146977, a new potent nonpeptide tachykinin NK_3 receptor antagonist. Can J Physiol Pharmacol 2002; 80 (5): 482-8.

[13] Hunnun MJ, Brownschidle LA, Tian Y, Whitty CJ, Poosch MS, D'sa C, *et al.* Neurokinin-3 receptors modulate dopamine cell function and alter the effects of 6-hydroxydopamine. Brain Res 1995; 695 (1): 19-24.

[14] Naliviko E, Michaud JC, Soubrie P, Le Fur G, Feltz P. Tachykinin neurokinin-1 and neurokinin-3 receptor-mediated responses in guinea-pig substantia nigra: an *in vitro* electrophysiological study. Neuroscience 1997; 78 (3): 745-57.

[15] Emonds-Alt X, Bichon D, Ducoux JP, Heaulme M, Miloux B, Poncelet M, *et al.* SR 142801, the first potent non-peptide antagonist of the tachykinin NK-3 receptor. Life Sci 1995; 56 (1): PL27-PL32.

[16] Rupniak NMJ, Kramer MS. Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK_1) antagonists. Trends Pharmacol Sci 1999; 20 (12): 485-90.

[17] Ljungdahl A, Hokfelt T, Nilsson G, Goldstein M. Distribution of substance P-like immunoreactivity in the central nervous system of the rat-II. Light microscopic localization in relation to catecholamine-containing neurons. Neuroscience, 1978. pp. 945-76.

[18] Nilsson G, Hokfelt T, Pernow B. Distribution of substance P-like immuno-reactivity in the rat central nervous system as revealed by immunohistochemistry. Med Biol 1974; 52 (6): 424-7.

[19] Nilsson L, Winblad B, Volkmann I, Alafuzoff I, Bergstrom L. Characterization and quantification of ^{125}I-Bolton Hunter substance P binding sites in human brain. Neurochem Int 1991; 18 (3): 399-404.

[20] Drevets WC. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. Curr Op Neurobiol 2001; 11 (2): 240-9.

[21] Drevets WC. Prefrontal cortical-amygdalar metabolism in major depression. Ann NY Acad Sci 1999; 877: 614-37.

[22] Shelline YI. Neuroimaging studies of mood disorder effects on the brain. Biol Psychiatry 2003; 54 (1): 338-52.

[23] Tooney Paul A, Au Gough G, Chahl Loris A. Tachykinin NK$_1$ and NK$_2$ receptors in the prefrontal cortex of the human brain. Clin Exp Pharmacol Physiol 2000; 27 (11): 947-9.

[24] Tooney Paul A, Au Gough G, Chahl Loris A. Localisation of tachykinin NK$_1$ and NK$_2$ receptors in the human prefrontal and visual cortex. Neurosci Lett 2000; 282 (3): 185-8.

[25] Mantyh PW, Hunt SP, Maggio JE. Substance P receptors: localization by light microscopic autoradiography in rat brain using [^{125}I]BSP as the radioligand. Brain Res 1984; 307 (1-2): 147-65.

[26] Rothman RB, Herkenham M, Perl CB, Liang T, Cascieri MA. Visualization of rat brain receptors for the neuropeptide, substance P. Brain Res 1984; 309 (1): 47-54.

[27] Kaneko T, Shigemoto R, Nakanishi S, Mizuno N. Morphological and chemical characteristics of substance P receptor-immunoreactive neurons in the rat neocortex. Neuroscience 1994; 60 (1): 199-211.

[28] Stacey AE, Woodhall GL, Jones RSG. Activation of neurokinin-1 receptors promotes GABA release at synapses in the rat entorhinal cortex. Neuroscience 2002; 115 (2): 575-86.

[29] Sakamoto N, Takatsuji K, Shiosaka S, Tohyama M. Evidence for the existence of substance P-like immunoreactive neurons in the human cerebral cortex: an immunohistochemical analysis. Brain Res 1985; 325 (1-2): 322-6.

[30] Iritani S, Fujii M, Satoh K. The distribution of substance P in the cerebral cortex and hippocampal formation: An immunohistochemical study in the monkey and rat. Brain Res Bull 1989; 22 (2): 295-303.

[31] Penny GR, Afsharpour S, Kitai ST. Substance P-immunoreactive neurons in the neocortex of the rat: a subset of the glutamic acid decarboxylase-immunoreactive neurons. Neurosci Lett 1986; 65 (1): 53-9.

[32] Sakanaka M, Shiosaka S, Takatsuki K, Tohyama M. Evidence for the existence of a substance P-containing pathway from the nucleus laterodorsalis tegmenti (Castaldi) to the medial frontal cortex of the rat. Brain Res 1983; 259 (1): 123-6.

[33] Vincent SR, Satoh K, Armstrong DM, Fibiger HC. Substance P in the ascending cholinergic reticular system. Nature 1983; 306 (5944): 688-91.

[34] Hunung JF, De Tribolet N, Tork I. Morphology and distribution of neuropeptide-containing neurons in human cerebral cortex. Neuroscience 1992; 51 (2): 361-75.

[35] Inkah RL, Goldman-Rakic P, Lennith C. Dual role of substance P/GABA axons in cortical neurotransmission: synaptic triads on pyramidal cell spines and basket-like innervation of layer II-III calbindin interneurons in primate prefrontal cortex. Cereb Cortex 1997; 7 (4): 359-73.

[36] Jones R, DeFelipe J, Hendry S, Maggio J. A study of tachykinin-immunoreactive neurons in monkey cerebral cortex. J. Neurosci. 1988; 8 (4): 1206-24.

[37] Stacey AE, Woodhall GL, Jones RS. Neurokinin-receptor-mediated depolarization of cortical neurons elicits an increase in glutamate release at excitatory synapses. Eur J Neurosci 2002; 16 (10): 1896-906.

[38] Jones RS, Olpe HR. Tachykinin responses in the rat cingulate cortex: a comparison of the effects of iontophoretically applied substance P and neurokinin A. Eur J Pharmacol 1985; 115 (1): 111-6.

[39] Jones RS, Mondadori C, Olpe HR. Neuronal sensitivity to substance P is increased after repeated treatment with tranylcypromine, carbamazepine or oxaprotiline, but decreased after repeated electroconvulsive shock. Neuropharmacology 1986; 24 (7): 627-33.

[40] Jones RS, Olpe HR. Monoaminergic modulation of the sensitivity of neurones in the cingulate cortex to iontophoretically applied substance P. Brain Res 1984; 311 (2): 297-305.

[41] Jones RS, Olpe HR. Activation of the noradrenergic projection from locus coeruleus reduces the excitatory responses of anterior cingulate cortical neurones to substance P. Neuroscience 1984; 13 (3): 819-25.

[42] Greenshaw AJ, Rao TS, Nazarali AJ, Baker GB, Coutts RT. Chronic effects of tranylcypromine and 4-fluorotranylcypromine on regional brain monoamine metabolism in rats: A comparison with clorgyline. Biol Psychiatry 1989; 25 (8): 1014-20.

[43] Overstreet DH. The Flinders sensitive line rats: a genetic animal model of depression. Neurosci Biobehav Rev 1993; 17 (1): 51-68.

[44] Burnet Philip WJ, Miller R, Lewis Louise J, Pei Q, Sharp T, Harrison Paul J. Electroconvulsive shock increases tachykinin NK$_1$ receptors, but not the encoding mRNA, in rat cortex. Eur J Pharmacol 2001; 413 (2-3): 213-9.

[45] Brodin K, Rosen A, Iwarsson K, Ogren SO, Brodin E. Increased levels of substance P and cholecystokinin in rat cerebral cortex following repeated electroconvulsive shock and subchronic treatment with a serotonin uptake inhibitor. Act Physiol Scand 1989; 136 (4): 613-4.

[46] Stenfors C, Hjallemp P, Mailie AA, Theodorsson E. Concurrent analysis of neuropeptides and biogenic amines in brain tissue of rats treated with electroconvulsive stimuli. Brain Res 1995; 698 (1-2): 39-45.

[47] Husum H, Vasquez PA, Mathe AA. Changed concentrations of tachykinins and neuropeptide Y in brain of a rat model of depression: lithium treatment normalizes tachykinins. Neuropsychopharmacology 2001; 24 (2): 183-91.

[48] Mantyh PW, Allen CJ, Ghilardi JR, Rogers SD, Mantyh CR, Liu H, *et al.* Rapid endocytosis of a G protein-coupled receptor: substance P evoked internalization of its receptor in the rat striatum *in vivo*. Proc Nat Acad Sci USA 1995; 92 (7): 2622-6.

[49] Mantyh PW, DeMaster E, Malhotra A, Ghilardi JR, Rogers SD, Mantyh CR, *et al.* Receptor endocytosis and dendrite reshaping in spinal neurons after somatosensory stimulation. Science 1995; 268 (5217): 1629-32.

[50] Hong JS, Tilson HA, Yoshikawa R. Effects of lithium and haloperidol administration on the rat brain levels of substance P. J Pharmacol Exp Ther 1983; 224 (3): 590-3.

[51] Davidson RJ, Lewis DA, Alloy LB, Amaral DG, Bush G, Cohen JD, *et al.* Neural and behavioral substrates of mood and mood regulation. Biol Psychiatry 2002; 52 (6): 478-502.

[52] LeDoux JE. Emotion circuits in the brain. Ann Rev Neurosci 2000; 23: 155-84.

[53] Shibata S, Yamashita K, Yamamoto R, Ozaki T, Ueki S. Effects of benzodiazepine and GABA antagonists on anticonflict effects of antianxiety drugs injected into the rat amygdala in a water-lick suppression test. Psychopharmacology 1989; 98 (1): 38-44.

[54] Gonzalez LE, Andrews N, File SE. 5-HT1A and benzodiazepine receptors in the basolateral amygdala modulate anxiety in the social interaction test, but not in the elevated plus-maze. Brain Res 1996; 732 (1-2): 145-53.

[55] Zangrossi H Jr, Viana MB, Graeff FG. Anxiolytic effect of intra-amygdala injection of midazolam and 8-hydroxy-2 (di-n-propylamino)tetralin in the elevated T-maze. Eur J Pharmacol 1999; 369 (3): 267-70.

[56] Duncan GE, Breese GR, Criswell H, Stumpf WE, Mueller RA, Covey JD. Effects of antidepressant drugs injected into the amygdala on behavioral responses of rats in the forced swim test. J Pharmacol Exp Ther 1986; 238 (2): 758-62.

[57] Roberts GW, Woodhams PL, Polak JM, Crow TJ. Distribution of neuropeptides in the limbic system of the rat: the amygdaloid complex. Neuroscience 1982; 7 (1): 99-131.

[58] Shimeda S, Inagaki S, Kubota Y, Ogawa N, Shiosaki T, Takagi H. Coexistence of peptides (corticotropin releasing factor/neurotensin and substance P/somatostatin) in the bed nucleus of the stria terminalis and central amygdaloid nucleus of the rat. Neuroscience 1989; 30 (2): 377-83.

[59] Gimson PC, Jessell T, Paxinos G, Cuello AC. Substance P in the amygdaloid complex, bed nucleus and stria terminalis of the rat brain. Brain Res 1978; 149 (1): 97-105.

[60] Wray S, Hoffman GE. Organization and interrelationship of neuropeptides in the central amygdaloid nucleus of the rat. Peptides 1983; 4 (4): 525-41.

[61] Cassell MD, Gray TS, Kiss JZ. Neuronal architecture in the rat central nucleus of the amygdala: a cytological, hodological, and immunocytochemical study. J Comp Neurol 1986; 246 (4): 478-99.

[62] Cassell MD, Gray TS. Morphology of peptide-immunoreactive neurons in the rat central nucleus of the amygdala. J Comp Neurol 1989; 281 (2): 320-33.

[63] Sakanaka M, Shiosaka S, Takatsuki K, Inagaki S, Takagi H, Senba E, *et al.* Experimental immunohistochemical studies on the amygdalofugal peptidergic (substance P and somatostatin) fibers in the stria terminalis of the rat. Brain Res 1981; 221 (2): 231-42.

[64] Maeno H, Kiyama H, Tohyama M. Distribution of the substance P receptor (NK-1 receptor) in the central nervous system. Brain Res Mol 1993; 18 (1-2): 43-58.

[65] Cooper PE, Fernstrom MH, Rorstad OP, Leeman SE, Martin JD. The regional distribution of somatostatin, substance P and neurotensin in human brain. Brain Res 1981; 218 (1-2): 219-32.

[66] Chawla MK, Chiuenza GM, Young WS 3rd, McMullen NT, Rance NE. Localization of neurons expressing substance P and neurokinin B gene transcripts in the human hypothalamus and basal forebrain. J Comp Neurol 1997; 384 (3): 429-42.

[67] Gale JS, Bird ED, Spokes EG, Iversen LL, Jessel T. Human brain substance P: distribution in controls and Huntington's chorea. J Neurochem 1978; 30 (3): 633-4.

[68] Benzing WC, Mufson EJ, Armstrong DM. Immunocytochemical distribution of peptidergic and cholinergic fibers in the human amygdala: their depletion in Alzheimer's disease and morphologic alteration in non-demented elderly with numerous senile plaques. Brain Res 1993; 625 (1): 135-48.

[69] Ciobanlotto L, Hurd YL, Murdock P, Wahlin JP, Melotto S, Corsi M, *et al.* Neurokinin 1 receptor and relative abundance of the short and long isoforms in the human brain. Eur J Neurosci 2003; 17 (9): 1736-46.

[70] Maubach KA, Martin K, Smith DW, Hewson L, Franklin PA, Harrison T, *et al.* Substance P stimulates inhibitory synaptic transmission in the guinea pig basolateral amygdala *in vitro*. Neuropharmacology 2001; 40 (6): 806-17.

[71] Le Gal, La Salle G, Ben-Ari Y. Microiontophoretic effects of substance P on neurons of the medial amygdala and putamen of the rat. Brain Res 1977; 135 (1): 171-9.

[72] Shimoyama Y, Misushio H, Takashima M, Ichikawa H, Takahashi K. Reduction of substance P after chronic antidepressants treatment in the striatum, substantia nigra and amygdala of the rat. Brain Res 1990; 739 (1-2): 70-8.

[73] Kendler KS, Karkowski LM, Prescott CA. Causal relationship between stressful life events and the onset of major depression.[comment]. Am J Psychiatry 1999; 156 (6): 837-41.

[74] Honkaniemi J, Colocalization of peptide- and tyrosine hydroxylase-like immunoreactivities with Fos-immunoreactive neurons in rat central amygdaloid nucleus after immobilization stress. Brain Res 1992; 598 (1-2): 107-13.

[75] Takayama H, Ota Z, Ogawa N. Effect of immobilization stress on neuropeptides and their receptors in rat central nervous system. Reg Peptides 1986; 15 (3): 239-48.

[76] Smith DW, Hewson L, Fuller P, Williams AR, Wheeldon A, Rupniak NM. The substance P antagonist L-760, 735 inhibits stress induced NK(1) receptor internalisation in the basolateral amygdala. Brain Res 1999; 848 (1-2): 90-3.

[77] Mulkwijk HE, Hartog K, van der Poel AM, Mos J, Olivier B. Reduction of guinea pig pup isolation calls by anxiolytic and antidepressant drugs. Psychopharmacology 1996; 128 (1): 31-8.

[78] Winslow JT, Insel TR. The infant rat separation paradigm: a novel test for novel anxiolytics. Trends Pharmacol Sci 1991; 12 (11): 402-4.

[79] Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, *et al.* Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 1998; 281 (5383): 1640-5.

[80] Bryce S, Smith D, Carlson E, Hewson L, Rigby M, O'Donnell R, Harrison T, Rupniak NM. Intra-amygdala injection of the substance P (NK$_1$ receptor) antagonist L-760735 inhibits neonatal vocalisations in guinea-pigs. Neuropharmacology 2001; 41 (1): 130-7.

[81] Rogers SD, Salak-Johnson JL, Schwen MJ, Pomonis JD, Mantyh PW. Reduced anxiety related behavior following ablation of amygdalu neurons expressing substance P receptor. Society for Neuroscience Abstracts 2000; 26 (1-2): Abstract No.

[82] Gray TS, Magnuson DJ. Peptide immunoreactive neurons in the amygdala and the bed nucleus of the stria terminalis project to the midbrain central gray in the rat. Peptides 1992; 13 (3): 451-60.

[83] De Araujo IE, Huston JP, Brandao ML. Place aversion induced by microinjections of C-fragment of substance P into the dorsal periaqueductal gray of rats is mediated by tachykinin NK$_1$ receptors. Peptides 2001; 22 (9): 1447-52.

[84] Aguiar MS, Brandao ML. Conditioned place aversion produced by microinjections of substance P into the periaqueductal gray of rats. Behav Pharmacol 1994; 5 (3): 369-73.

[85] Aguiar MS, Brandao ML. Effects of microinjections of the neuropeptide substance P in the dorsal periaqueductal gray on the behaviour of rats in the plus-maze test. Physiol Behav 1996; 60 (4): 1183-6.

[86] Monyenu R, De Oca BM, Fanselow MS, Marsden CA. Differential effects of neurokinin-1 receptor activation in subregions of the periaqueductal gray matter on conditional and unconditional fear behaviors in rats. Behav Neurosci 1998; 112 (5): 1125-33.

[87] Commons, KG, Valentino RJ. Cellular basis for the effects of substance P in the periaqueductal gray and dorsal raphe nucleus. J Comp Neurol 2002; 447 (1): 82-97.

[88] Bowker RM, Westlund KN, Sullivan MC, Wilber JF, Coulter JD. Descending serotonergic, peptidergic and cholinergic pathways from the raphe nuclei: a multiple-transmitter complex. Brain Res 1983; 288 (1-2): 33-48.

[89] Hokfelt T, Ljungdahl A, Steinbusch H, Verhofstad A, Nilsson G, Brodin E, *et al.* Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine-containing neurons in the rat central nervous system. Neuroscience 1978; 3 (6): 517-38.

[90] Chan-Palay V. Evidence for the coexistence of serotonin and substance P in single raphe cells and fiber plexuses: combined immunocytochemistry and autoradiography. Adv Exp Med Biol 1981; 133: 81-97.

[91] Cuello AC, Jessell TM, Kanazawa I, Iversen LL. Substance P: localization in synaptic vesicles in rat central nervous system. J Neurochem 1977; 29 (4): 747-51.

[92] Neckers LM, Schwartz JP, Wyatt RJ, Speciale SG. Substance P afferents from the habenula innervate the dorsal raphe nucleus. Exp Brain Res 1979; 37 (3): 619-23.

[93] Magoul R, Onteniente B, Ohlin A, Calas A. Inter- and intracellular relationship of substance P-containing neurons with serotonin and GABA in the dorsal raphe nucleus: combination of autoradiographic and immunocytochemical techniques. J Histochem Cytochem 1986; 34 (6): 735-42.

[94] Ma QP, Bleasdale C. Modulation of brain stem monoamines and gamma-aminobutyric acid by NK$_1$ receptors in rats. Neuroreport 2002; 13 (14): 1809-12.

[95] Saffroy M, Beaujouan J-C, Torrens Y, Besseyre J, Bergstrom L, Glowinski J. Localization of tachykinin binding sites (NK$_1$, NK$_2$, NK$_3$ ligands) in the rat brain. Peptides 1988; 9 (2): 227-41.

[96] Beaujouan JC, Torrens Y, Saffroy M, Glowinski J. Quantitative autoradiographic analysis of the distribution of binding sites for [^{125}I]Bolton Hunter derivatives of eledoisin and substance P in the rat brain. Neuroscience 1986; 18 (4): 857-75.

[97] Nakaya Y, Kaneko T, Shigemoto R, Nakanishi S, Mizuno N. Immunohistochemical localization of substance P receptor in the central nervous system of the adult rat. J Comp Neurol 1994; 347 (2): 249-74.

[98] Lieget S, Clay N, Cespuglio R. Neurokinin-3 (NK$_3$)- and NK$_2$-immunoreactive neurons in serotonergic cell groups in the rat brain. Neurosci Lett 2002; 323 (2): 146-50.

[99] Froger N, Gardier AM, Moratalla R, Alberti I, Lena I, Boni C, *et al.* 5-hydroxytryptamine (5-HT)1A autoreceptor adaptive changes in substance P (neurokinin 1) receptor knock-out mice mimic antidepressant-induced desensitization. J Neurosci 2001; 21 (20): 8188-97.

[100] Sergeyev V, Hokfelt T, Hurd Y. Serotonin and substance P co-exist in dorsal raphe neurons of the human brain. Neuroreport 1999; 10 (18): 3967-70.

[101] Baker KG, Halliday GM, Hornung JP, Geffen LB, Cotton RG, Tork I. Distribution, morphology and number of monoamine-synthesizing and substance P-containing neurons in the human dorsal raphe nucleus. Neuroscience 1991; 42 (3): 757-75.

[102] Charara A, Parent A. Chemoarchitecture of the primate dorsal raphe nucleus. J Chem Anat 1998; 15 (2): 111-27.

[103] Conley RK, Cumbelbatch MJ, Mason GS, Williamson DJ, Harrison T, Tucker K, *et al.* Substance P (neurokinin 1) receptor antagonists enhance dorsal raphe neuronal activity. J Neurosci 2002; 22 (17): 7730-6.

[104] Appell KC, Fragale BJ, Lincig J, Singh S, Tomonik BE. Antagonists that demonstrate species differences in neurokinin-1 receptors. Mol Pharmacol 1992; 41 (4): 772-8.

[105] Sachais BS, Snider RM, Lowe JA 3rd, Krause JE. Molecular basis for the species selectivity of the substance P antagonist CP-96, 345. J Biol Chem 1993; 268 (4): 2319-23.

[106] Wang RY, Aghajanian GK. Physiological evidence for habenula as major link between forebrain and midbrain raphe. Science 1977; 197 (4298): 89-91.

[107] Liu R, Ding Y, Aghajanian GK. Neurokinins activate local glutamatergic inputs to serotonergic neurons of the dorsal raphe nucleus. Neuropsychopharmacology 2002; 27 (3): 329-40.

[108] Valentino RJ, Bey V, Pernar L, Commons KG. Substance P acts through local circuits within the rat dorsal raphe nucleus to alter serotonergic neuronal activity. J Neurosci 2003; 23 (18): 7155-9.

[109] Santarelli L, Gobbi G, Debs PC, Sibille EL, Escorbin P, Hen R, *et al*. Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. Proc Natl Acad Sci USA 2001; 98 (4): 1912-7.

[110] Haddjeri N, Blier P. Sustained blockade of neurokinin-1 receptors enhances serotonin neurotransmission. Biol Psychiatry 2001; 50 (3): 191-9.

[111] Vandermaelen CP, Aghajanian GK. Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. Brain Res 1983; 289 (1-2): 109-19.

[112] Pineyro G, Blier P. Autoregulation of serotonin neurons: role in antidepressant drug action. Pharmacol Rev 1999; 51 (3): 533-91.

[113] Fornal CA, Metzler CW, Gallegos RA, Veasey SC, McCreary AC, Jacobs BL. WAY-100635, a potent and selective 5-hydroxytryptamine1A antagonist, increases serotonergic neuronal activity in behaving cats: comparison with (S)-WAY-100135. J Pharmacol Exp Ther 1996; 278 (2): 752-62.

[114] Millan MJ, Lejeune F, De Nanteuil G, Gobert A. Selective blockade of neurokinin (NK)(1) receptors facilitates the activity of mesencephalic pathways projecting to frontal cortex and dorsal hippocampus in rats. J Neurochem 2001; 76 (6): 1949-54.

[115] Zocchi A, Varnier G, Arban R, Griffante C, Zanetti L, Bettelini L, *et al*. Effects of antidepressant drugs and GR 205171, an neurokinin-1 (NK_1) receptor antagonist, on the response in the forced swim test and on monoamine extracellular levels in the frontal cortex of the mouse. Neurosci Lett 2003; 345 (2): 73-6.

[116] Ase AR, Reader TA, Hen R, Riad M, Descarries L. Altered serotonin and dopamine metabolism in the CNS of serotonin 5-HT(1A) or 5-HT(1B) receptor knockout mice. J Neurochem 2000; 75 (6): 2415-26.

[117] Commons KG, Connolley KR, Valentino RJ. A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders. Neuropsychopharmacology 2003; 28 (2): 206-15.

[118] Anand A, Charney DS. Norepinephrine dysfunction in depression. J Clin Psychiatry 2000; 61 (Suppl 10): 16-24.

[119] Pmzer A. Norepinephrine involvement in antidepressant action. J Clin Psychiatry 2000; 61 (Suppl 10): 25-30.

[120] Leonard BE. The role of noradrenaline in depression: a review. J Psychopharmacol 1997; 11 (4 Suppl): S39-47.

[121] Pammer C, Gorcs T, Palkovits M. Peptidergic innervation of the locus coeruleus cells in the human brain. Brain Res 1990; 515 (1-2): 247-55.

[122] Nomura H, Shiosaka S, Tohyama M. Distribution of substance P-like immunoreactive structures in the brainstem of the adult human brain: an immunocytochemical study. Brain Res 1987; 404 (1-2): 365-70.

[123] Fodor M, Gorcs TJ, Palkovits M. Immunohistochemical study on the distribution of neuropeptides within the pontine tegmentum - Particularly the parabrachial nuclei and the locus coeruleus of the human brain. Neuroscience 1992; 46 (4): 891-908.

[124] Jordan D, Kummli I, Rambaud C, Gilly R, Bouvier R, Kopp N. Regional distribution of substance P-binding sites in the brainstem of the human newborn. Brain Res 1995; 695 (2): 117-24.

[125] Ljungdahl A, Hokfelt T, Nilsson G, Goldstein M. Distribution of substance P-like immunoreactivity in the central nervous system of the rat--II. Light microscopic localization in relation to catecholamine-containing neurons. Neuroscience 1978; 3 (10): 945-76.

[126] Pickel VM, Joh TH, Reis DJ, Leeman SE, Miller RJ. Electron microscopic localization of substance P and enkephalin in axon terminals related to dendrites of catecholaminergic neurons. Brain Res 1979; 160 (3): 387-400.

[127] Shults CW, Quirion R, Chronwall B, Chase TN, O'Donohue TL. A comparison of the anatomical distribution of substance P and substance P receptors in the rat central nervous system. Peptides 1984; 5 (6): 1097-128.

[128] McLean S, Ganong AH, Seeger TF, Bryce DK, Fran KG, Reynolds LS, *et al*. Activity and distribution of binding sites in brain of a nonpeptide substance P (NK_1) receptor antagonist. Science 1991; 251 (4992): 437-9.

[129] Shults CW, Quirion R, Jansen RT, Moody TW, O'Donohue TL, Chase TN. Autoradiographic localization of substance P receptors using ^{125}I-substance P. Peptides 1982; 3 (6): 1073-5.

[130] Quirion R, Shults CW, Moody TW, Pert CB, Chase TN, O'Donohue TL. Autoradiographic distribution of substance P receptors in rat central nervous system. Nature 1983; 303 (5919): 714-6.

[131] Chen I.-W, Wei L-C, Liu H-L, Rao Z-R. Noradrenergic neurons expressing substance P receptor (NK_1) in the locus coeruleus complex: a double immunofluorescence study in the rat. Brain Res 2000; 877 (1): 155-9.

[132] Hahn MK, Bunnin MJ. Stress-induced c-fos expression in the rat locus coeruleus is dependent on neurokinin 1 receptor activation. Neuroscience 1999; 94 (4): 1183-8.

[133] Guyenet PG, Aghajanian GK. ACh, substance P and met-enkephalin in the locus coeruleus: Pharmacological evidence for independent sites of action. Eur J Pharmacol 1979; 53 (4): 319-28.

[134] Guyenet PG, Aghajanian GK. Excitation of neurons in the nucleus locus coeruleus by substance P and related peptides. Brain Res 1977; 136 (1): 178-84.

[135] Cherubini E, Pinnock RD, Henderson G. Substance P excitation of rat locus coeruleus neurones. Eur J Pharmacol 1983; 94 (1-2): 93-9.

[136] McLean S, Ganong A, Seymour PA, Snider RM, Desai MC, Rosen T, *et al*. Pharmacology of CP-99, 994: a nonpeptide antagonist of the tachykinin neurokinin-1 receptor. J Pharmacol Exp Ther 1993; 267 (1): 472-9.

[137] Steinberg R, Alonso R, Rouquier L, Desvignes C, Michaud J-C, Cudennec A, *et al*. SSR240600 ((R)-2-(1-(2-(4-(2-(3, 5-bis(trifluoromethyl) phenyl)acetyl) 2 (3, 4-dichlorophenyl)-2-morpholinyl) ethyl)-4-piperidinyl)-2-methylpropanamide), a centrally active nonpeptide antagonist of the tachykinin neurokinin 1 receptor. II. Neurochemical and behavioral characterization. J Pharmacol Exp Ther 2002; 303 (3): 1160-8.

[138] Berrard L, Rouleau D, Dougault I, Allouard N, Le-Fur G, Soubrie P, *et al*. Permissive role of neurokinin NK_1 receptors in NK_1 receptor-mediated activation of the locus coeruleus revealed by SR 142801. Synapse 2002; 43 (1): 62-9.

[139] Haddjeri N, Blier P. Effect of neurokinin-1 receptor antagonists on the function of 5-HT and noradrenaline neurons. Neuroreport 2000; 11 (6): 1323-7.

[140] Meubach KA, Martin K, Chicchi G, Harrison T, Wheeldon A, Swain CJ, *et al*. Chronic substance P (NK_1) receptor antagonist and conventional antidepressant treatment increases burst firing of monoamine neurones in the locus coeruleus. Neuroscience 2002; 109 (3): 609-17.

[141] Teixeira Raquel M, Santos Adair RS, Ribeiro Sandro J, Calixto Joao B, Rae Giles A, De Lima Thereza CM. Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze behavior in mice. Eur J Pharmacol 1996; 311 (1): 7-14.

[142] Gavioli EC, Canteras NS, De Lima TC. The role of lateral septal NK_1 receptors in mediating anxiogenic effects induced by intracerebroventricular injection of substance P. Behav Brain Res 2002; 134 (1-2): 411-5.

[143] Gavioli EC, Canteras NS, De Lima TCM. Anxiogenic-like effect induced by substance P injected into the lateral septal nucleus. Neuroreport 1999; 10 (16): 3399-403.

[144] Uaresta IP, Assreuy J, De Lima TC. Nitric oxide involvement in the anxiogenic-like effect of substance P. Behav Brain Res 2001; 121 (1-2): 199-205.

[145] De Araujo JE, Silva RCD, Huston JP, Brandao ML. Anxiogenic effects of substance P and its 7-11 C terminal, but not the 1-7 N terminal, injected into the dorsal periaqueductal gray. Peptides 1999; 20 (12): 1437-43.

[146] De Araujo JE, Huston JP, Brandao ML. Opposite effects of substance P fragments C (anxiogenic) and N (anxiolytic) injected into dorsal periaqueductal gray. Eur J Pharmacol 2001; 412 (1): 43-51.

[147] Barros M, De Souza Silva MA, Huston JP, Tomaz C. Anxiolytic-like effects of substance P fragment (SP(1-7)) in non-human primates (Callithrix penicillata). Peptides 2002; 23 (5): 967-73.

[148] Bchevelny MB, Hasenohrl RU, Huston JP, Tomaz C. Comparison of neurokinin SP with diazepam in effects on memory and fear parameters in the elevated T-maze free exploration paradigm. Peptides 2001; 22 (7): 1031-6.

[149] Nikolaus S, Huston JP, Hasenohrl RU. The neurokinin-1 receptor antagonist WIN51, 708 attenuates the anxiolytic like effects of ventral pallidal substance P injection. Neuroreport 1999; 10 (11): 2293-6.

[150] Hasenohrl RU, Jenjjans U, De Souza Silva MA, Tomaz C, Huston JP. Anxiolytic-like action of neurokinin substance P administered systemically or into the nucleus basalis magnocellularis region. Eur J Pharmacol 1998; 354 (2-3): 123-33.

[151] Belzung C, Griebel G. Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav Brain Res 2001; 125 (1-2): 141-9.

[152] Millan MJ. The neurobiology and control of anxious states. Prog Neurobiol 2003; 70 (2): 83-244.

[153] Smith DG, Iyengar S. Anxiolytic - Like Effects In Gerbils in the Elevated Plus Maze. Abs Soc Neurosci 2002; 306.

[154] Varty GB, Morgan CA, Cohen-Williams ME, Coffin VL, Carey GJ. The gerbil elevated plus-maze: I. behavioral characterization and pharmacological validation. Neuropsychopharmacology 2002; 27 (3): 357-70.

[155] Rupniak NM, Carlson EJ, Webb JK, Harrison T, Porsolt RD, Roux S, *et al.* Comparisons of the phenotype of NK1R-/- mice with pharmacological blockade of the substance P (NK1) receptor in assays for antidepressant and anxiolytic drugs. Behav Pharmacol 2001; pp. 497-508.

[156] Murtra P, Sheasby AM, Hunt SP, De Felipe C. Rewarding effects of opiates are absent in mice lacking the receptor for substance P. Nature 2000; 405 (6783): 180-3.

[157] Varnout A, Veenstra S, Hauser R, Ofner S, Ruppan P, Schilling W, *et al.* NKP608: a selective NK-1 receptor antagonist with anxiolytic-like effects in the social interaction and social exploration test in rats. Reg Peptides 2000; 96 (1-2): 7-16.

[158] Gentsch C, Cutler M, Vassout A, Veenstra S, Ruppan P. Anxiolytic effect of NKP608, a NK1-receptor antagonist, in the social investigation test in gerbils. Behav Brain Res 2002; 133 (2): 363-8.

[159] File SE. NKP608, an NK1 receptor antagonist, has an anxiolytic action in the social interaction test in rats. Psychopharmacology 2000; 152 (1): 105-9.

[160] Cheeta S, Tucci S, Sandhu J, Williams AR, Rupniak NM, File SE. Anxiolytic actions of the substance P (NK1) receptor antagonist L-760735 and the 5-HT1A agonist, 8-OH-DPAT in the social interaction test in gerbils. Brain Res 2001; 915 (2): 170-5.

[161] File SE. Anxiolytic action of a neurokinin1 receptor antagonist in the social interaction test. Pharmacol Biochem Behav 1997; 58 (3): 747-52.

[162] Ribeiro Rafinha F, De Lima Thereza CM. Participation of GABAA receptors in the modulation of experimental anxiety by tachykinin agonists and antagonists in mice. Prog Neuropsychopharm Biol Psychiat 2002; 26 (5): 861-9.

[163] Rupniak NM, Carlson EC, Harrison T, Oates B, Seward E, Owen S, *et al.* Pharmacological blockade or genetic deletion of substance P (NK1) receptors attenuates neonatal vocalisation in guinea-pigs and mice. Neuropharmacology 2000; 39 (8): 1413-21.

[164] Kramer M, Cutler N, Feighner J, Shrivastava R, Carman J, Liu G, Snively D, *et al.* Demonstration that the novel substance P NK1 receptor antagonist MK-869 is an effective antidepressant. Society for Neuroscience Abstracts 1998; 24 (1-2): 1490.

[165] Newman JD, Bachevalier J. Neonatal ablations of the amygdala and inferior temporal cortex alter the vocal response to social separation in rhesus macaques. Brain Res 1997; 758 (1-2): 120-6.

[166] Dalvi A, Lucki I. Murine models of depression [comment]. Psychopharmacology 1999; 147 (1): 14-6.

[167] Dilkel-Gorro A, Raez I, Michiel K, Zimmer A. Diminished anxiety- and depression-related behaviors in mice with selective deletion of the Tac1 gene. J Neurosci 2002; 22 (32): 10046-52.

[168] Rupniak NMJ, Carlson EJ, Webb JK, Harrison T, Porsolt RD, Roux S, *et al.* Comparison of the phenotype of NK1R -/- mice with pharmacological blockade of the substance P (NK1) receptor in assays for antidepressant and anxiolytic drugs. Behav Pharmacol 2001; 12 (6-7): 497-508.

[169] Varty GB, Cohen-Williams ME, Hunter JC. The antidepressant-like effects of neurokinin NK1 receptor antagonists in a gerbil tail suspension test. Behav Pharmacol 2003; 14 (1): 87-95.

[170] Muscat R, Papp M, Willner P. Reversal of stress-induced anhedonia by the atypical antidepressants fluoxetine and maprotiline. Psychopharmacology 1992; 109 (4): 433-8.

[171] Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci Biobehav Rev 1992; 16 (4): 525-34.

[172] Papp M, Vassout A, Gentsch C. The NK1-receptor antagonist NKP608 has an antidepressant-like effect in the chronic mild stress model of depression in rats. Behav Brain Res 2000; 115 (1): 19-23.

[173] McEwen BS. Stress and hippocampal plasticity. Ann Rev Neurosci 1999; 22: 105-22.

[174] Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, *et al.* Requirement of Hippocampal Neurogenesis for the Behavioral Effects of Antidepressants. Science 2003; 301 (5634): 805-9.

[175] Lee HJ, Kim JW, Yim SV, Kim MJ, Kim SA, Kim YJ, *et al.* Fluoxetine enhances cell proliferation and prevents apoptosis in dentate gyrus of maternally separated rats. Mol Psychiatry 2001; 6 (6): 610, 725-8.

[176] van Kampen M, Kramer M, Hiemke C, Flugge G, Fuchs E. The chronic psychosocial stress paradigm in male tree shrews: evaluation of a novel animal model for depressive disorders. Stress 2002; 5 (1): 37-46.

[177] van der Hart MGC, Czeh B, de Biurrun G, Michaelis T, Watanabe T, Natt O, *et al.* Substance P receptor antagonist and clomipramine prevent stress-induced alterations in cortical metabolites, cytogenesis in the dentate gyrus and hippocampal volume. Mol Psychiatry; 7 (9): 933-41.

[178] Mohammda S, Harris EA, Sheasby A, de Felipe C, Mantyh PW, Rupniak NMJ, *et al.* Adult Neurogenesis Is Increased in the Hippocampus of NK1 Receptor Knockout Mice. Society for Neuroscience Abstracts 2002; 23.

[179] Schedlowski M, Fluge T, Richter S, Tewes U, Schmidt RE, Wagner TO. Beta-endorphin, but not substance-P, is increased by acute stress in humans. Psychoneuroendocrinology 1995; 20 (1): 103-10.

[180] Weiss DW, Hirt R, Tarcic N, Beryim Y, Ben-Zur H, Dreznitz S, *et al.* Studies in psychoneuroimmunology: psychological, immunological, and neuroendocrinological parameters in Israeli civilians during and after a period of Scud missile attacks. Behav Med 1996; 22 (1): 3-14.

[181] Rimon R, Le Greves P, Nyberg F, Heikkila L, Salmela L, Terenius L. Elevation of substance P-like peptides in the CSF of psychiatric patients. Biol Psychiatry 1984; 19 (4): 509-16.

[182] Heikkila L, Rimon R, Terenius L. Dynorphin A and substance P in the cerebrospinal fluid of schizophrenic patients. Psychiat Res 1990; 34 (3): 229-36.

[183] Berrettini WH, Rubinow DR, Nurnberger JI Jr, Simmons-Alling S, Post RM, Gershon ES. CSF substance P immunoreactivity in affective disorders. Biol Psychiatry 1985; 20 (9): 965-70.

[184] Toresson G, Brodin E, de las Carreras C, Nordin C, Zachau AC, Bertilsson L. Quantitation of N-terminally extended tachykinins in cerebrospinal fluid from healthy subjects. Reg Peptides 1994; 50 (2): 185-91.

[185] Toresson G, Brodin E, Wahlstrom A, Bertilsson L. Detection of N-terminally extended substance P but not of substance P in human cerebrospinal fluid: quantitation with HPLC-radioimmunoassay. J Neurochem 1988; 50 (6): 1701-7.

[186] Mårtensson B, Nyberg S, Toresson G, Brodin E, Bertilsson L. Fluoxetine treatment of depression. Clinical effects, drug concentrations and immunoassay metabolites and N-terminally extended substance P in cerebrospinal fluid. Acta Psychiat Scand 1989; 79 (6): 586-96.

[187] Liu Z, Wells M, Dragee B, Nyberg F. A high-recovery extraction procedure for quantitative analysis of substance P and opioid peptides in human cerebrospinal fluid. Peptides 2000; 21 (6): 853-60.

[188] Russell IJ. The promise of substance P inhibitors in fibromyalgia. Rheumatic Diseases Clinics of North America 2002; 28 (2): 329-42.

[189] Dondy B, Baghai TC, Minov C, Schule C, Schwarz MJ, Zwanzger P, *et al.* Substance P serum levels are increased in major

depression: preliminary results. Biol Psychiatry 2003; 53 (6): 518-42.

[190] Clark JW, Senanayake PD, Solomon GD, Gallagher C. Substance P: correlation of CSF and plasma levels. Headache 1994; 34 (5): 261-4.

[191] Lieb K, Ahlvers K, Dziobek K, Strohbusch S, Reincke M, Feige B, *et al.* Effects of the neuropeptide substance P on sleep, mood, and neuroendocrine measures in healthy young men. Neuropsychopharmacology 2002; 27 (6): 1041-9.

[192] Burnet PW, Harrison PJ. Substance P (NK$_1$) receptors in the cingulate cortex in unipolar and bipolar mood disorder and schizophrenia. Biol Psychiatry 2000; 47 (1): 80-3.

[193] Stocknieier CA, Shi X, Rupniak L, Owellaiser JC, Jurjus G, Meltzer HY, *et al.* Neurokinin-1 receptors are decreased in major depressive disorder. Neuroreport 2002; 13 (9): 1223-7.

[194] Kramer MS, Winokur A, Kelsey J, Preskorn SH, Rothschild AJ, Snavely D, *et al.* Demonstration of the efficacy and safety of a novel substance P (NK$_1$) receptor antagonist in major depression. Neuropsychopharmacology 2004; 29 (2): 385-92.

[195] Ranga K, Krishnan R. Clinical experience with substance P receptor (NK$_1$) antagonists in depression. J Clin Psychiatry 2002; 63 (Suppl 11): 23-9.

[196] Kramer M. Clinical Update: substance P antagonists in patients with major depression. Assoc Eur Psychiat 2002.

[197] Chappell P. Effects of CP-122, 721, a selective NK-1 receptor antagonist, in patients with MDD. Presented at NCDEU June 12 2002.

[198] Hargreaves R. Imaging substance P receptors (NK$_1$) in the living human brain using positron emission tomography. J Clin Psychiatry 2002; 63 (Suppl 11): 18-24.

[199] Khan A, Khan SR, Walens G, Kolts R, Giller EL. Frequency of positive studies among fixed and flexible dose antidepressant clinical trials: an analysis of the food and drug administration summary basis of approval reports. Neuropsychopharmacology 2003; 28 (3): 552-7.

[200] Varty GB, Cohen-Williams ME, Morgan CA, Pylak U, Duffy RA, Lachowicz JE, *et al.* The gerbil elevated plus-maze II: anxiolytic-like effects of selective neurokinin NK$_1$ receptor antagonists. Neuropsychopharmacology 2002; 27 (3): 371-9.

[201] Rupniak NM, Webb JK, Fisher A, Smith D, Boyce S. The substance P (NK$_1$) receptor antagonist L-760735 inhibits fear conditioning in gerbils. Neuropharmacology 2003; 44 (4): 516-23.

[202] Casteleri MA, Liang T. Characterization of the substance P receptor in rat brain cortex membranes and the inhibition of radioligand binding by guanine nucleotides. J Biol Chem 1983; 258 (8): 5158-64.

[203] Lavielle S, Chassaing G, Ploux O, Loeuillet D, Rossignol J, Julien S, *et al.* Analysis of tachykinin binding site interactions using constrained analogues of tachykinins. Biochem Pharmacol 1988; 37 (1): 41-9.

[204] Viger A, Beaujouan JC, Torrens Y, Glowinski J. Specific binding of a ^{125}I-substance P derivative to rat brain synaptosomes. J Neurochem 1983; 40 (4): 1030-9.

[205] Igic R, Behnke R. Properties and distribution of angiotensin I converting enzyme. Curr Pharm Design 2003; 9(9): 697-706.

[206] van Waarde A, Vaalburg W, Doze P, Bosker FJ, Elsinga PH, PET imaging of brain neuroreceptors in human brain: a realistic goal or a mirage? Curr Pharm Design 2004; 10(13): 1519-30.

[207] Yoshikawa M, Takahashi M, Yang S. Delta-opioid peptides derived from plant proteins. Curr Pharm Design 2003; 9(16): 1325-30.

Cerebral Blood Flow Changes After Treatment of Social Phobia with the Neurokinin-1 Antagonist GR205171, Citalopram, or Placebo

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Background: *Evidence is accumulating that pharmacological blockade of the substance P preferring neurokinin-1 (NK1) receptor reduces anxiety. This study compared the effects of an NK1 receptor antagonist, citalopram, and placebo on brain activity and anxiety symptoms in social phobia.*

Methods: *Thirty-six patients diagnosed with social phobia were treated for 6 weeks with the NK1 antagonist GR205171 (5 mg), citalopram (40 mg), or matching placebo under randomized double-blind conditions. GR205171 was administered for 4 weeks preceded by 2 weeks of placebo. Before and after treatment, regional cerebral blood flow (rCBF) during a stressful public speaking task was assessed using oxygen-15 positron emission tomography. Response rate was determined by the Clinical Global Impression Improvement Scale.*

Results: *Patients improved to a larger extent with the NK1 antagonist (41.7% responders) and citalopram (50% responders), compared with placebo (8.3% responders). Within- and between-group comparisons showed that symptom improvement was paralleled by a significantly reduced rCBF response to public speaking in the rhinal cortex, amygdala, and parahippocampal-hippocampal regions. The rCBF pattern was corroborated in follow-up analyses of responders and subjects showing large state anxiety reduction.*

Conclusions: *Short-term administration of GR205171 and citalopram alleviated social anxiety. Neurokinin-1 antagonists may act like serotonin reuptake inhibitors by attenuating neural activity in a medial temporal lobe network.*

Key Words: Brain, NK1 antagonist, rCBF, social anxiety, SSRI, substance P

Peptide neurotransmitters like substance P (SP) have recently attracted considerable interest in the field of anxiety (Griebel 1999). For example, it has been demonstrated that pharmacological blockade of the SP preferring neurokinin-1 (NK1) receptor yields significant antianxiety and antidepressant effects in patients suffering from major depression (Kramer et al 1998, 2004). In animals, NK1 receptor antagonists have an anxiolytic profile in various models of anxiety such as the rat elevated plus maze, social interaction test, and tests of transient maternal separation (File 2000; Kramer et al 1998; Varty et al 2002). Genetic disruption of the NK1 receptor in mice also reduces anxiety and stress-related behaviors (Santarelli et al 2001). Intracerebral injections of SP agonists provoke anxiety in animal trials (Aguiar and Brandao 1996; Kramer et al 1998; Krase et al 1994), whereas administration of SP antagonists have anxiolytic effects (File 1997; Teixeira et al 1996). Moreover, in rats, central SP is released during aversive or noxious conditions (Brodin et al 1994; Rupniak and Kramer 1999). Thus, it has been proposed that anxiety is associated with increased levels of central SP (Hasenohrl et al 2000).

Kramer et al (1998) reported that the anxiolytic and antidepressant effects of an NK1 receptor antagonist were comparable to those produced by a selective serotonin reuptake inhibitor (SSRI). Selective serotonin reuptake inhibitors have rapidly become the pharmacological treatment of choice for major depressive disorder and also for various anxiety conditions (Gorman and Kent 1999). For instance, several placebo-controlled studies have shown that SSRIs are effective in social phobia, also known as social anxiety disorder (Van Ameringen et al 1999). This is a highly common (Furmark 2002), disabling (Wittchen et al 2000), and enduring (Yonkers et al 2001) condition, characterized by a fear of scrutiny or humiliation in social performance and interactional situations. Even though current pharmacological treatments of social phobia are helpful, they often produce only partial improvement (Ameringen et al 2000). A better understanding of the neurofunctional changes that underlie the beneficial effects on mood and anxiety could facilitate the development of new anxiolytic agents. The drug-brain interaction can be studied by functional neuroimaging techniques, such as position emission tomography (PET) and functional magnetic resonance imaging (fMRI).

Recently, we reported that citalopram and cognitive-behavioral therapy significantly reduced public speaking anxiety in patients with social phobia and that symptom improvement was associated with reduced regional cerebral blood flow (rCBF) mainly in the medial temporal lobe (MTL), including the amygdala, hippocampus, and the surrounding rhinal and parahippocampal cortices (Furmark et al 2002). Congruently, it has been observed that citalopram reduces resting-state neuronal activity in the left temporal cortex in patients with social phobia (van der Linden et al 2000). Several neuroimaging studies also point to a pivotal role for the MTL in the modulation of social anxiety. For instance, we showed that rCBF in the amygdaloid complex increased significantly more in patients with social phobia than

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Received January 6, 2005; revised March 7, 2005; accepted March 15, 2005.

0006-3223/05/$30.00
doi:10.1016/j.biopsych.2005.03.029

- Medial Temporal Lobe -

in unanxious control subjects during anxiety induced by a public speaking task (Tillfors et al 2001). Further, increased neural activity was observed in the left amygdaloid-hippocampal region and inferior temporal cortex during anticipation of the speaking task (Tillfors et al 2002). Activation of the MTL during speech anticipatory social anxiety was recently confirmed in a fMRI study (Lorberbaum et al 2004). Moreover, fMRI studies have reported increased amygdala and hippocampal activation during aversive conditioning (Schneider et al 1999) and enhanced amygdalar reactivity to social cues such as neutral faces in patients with social phobia, relative to control subjects (Birbaumer et al 1998; Veit et al 2002). Stein et al (2002) demonstrated that the MTL, including the amygdala, was more reactive to angry and contemptuous facial expressions than to happy or neutral expressions in patients with generalized social phobia compared with healthy control subjects. These neuroimaging results are, in turn, consistent with a wealth of data from animals and humans indicating that the MTL, especially the amygdala and hippocampus, is crucially involved in the regulation of anxiety-related behaviors (Davidson et al 2000; Davis and Whalen 2001; Gray and McNaughton 1996; LeDoux 1996, 2000).

Neurobiological data imply that the MTL is a potential target for SSRIs in the treatment of anxiety disorders. Neurokinin-1 antagonists may also act at the MTL level, since NK1 receptors are highly expressed in the amygdala and hippocampus (McLean et al 1991). In mammals, psychological stress such as maternal separation cause a release of SP in the amygdala (Kramer et al 1998), whereas anxiolytic and antidepressant drugs reduce central levels of SP, e.g., in the amygdala and hippocampus (Hasenohrl et al 2000; Shirayama et al 1996). However, it remains to be elucidated whether NK1 receptor antagonists are effective in the treatment of anxiety disorders in humans and whether these drugs act on unique or common neural networks compared with the SSRIs.

GR205171 is a selective NK1 receptor antagonist developed by GlaxoSmithKline (Gardner et al 1996). The GR205171 compound has shown good penetration to the brain and high affinity to NK1 receptors in rats (Rupniak et al 2003). Evaluation of GR205171 binding kinetic in monkeys, using PET, has confirmed the possibility to achieve high levels (>90%) of central NK1 receptor occupancy (Zamuner et al 2002). The aim of the present experimental study was to evaluate the effects of short-term treatment with GR205171, compared with a SSRI, on brain activity (rCBF) in patients diagnosed with social phobia. Patients received daily doses of GR205171, citalopram, or placebo under randomized and double-blind conditions during a 6-week period. Before and after this period, patients were exposed to a stressful public speaking task during which alterations in rCBF were studied by means of PET and oxygen-15 (^{15}O) labeled water. We hypothesized that anxiety reduction, following active drug administration, would be associated with decreased neural activity in the MTL region.

Methods and Materials

Screening

Participants were recruited through newspaper advertising. Initial screening included a brief telephone interview and social anxiety questionnaires returned by mail. Structured clinical diagnostic interviews (Structured Clinical Interview for DSM-IV [SCID]) (First et al 1998) were thereafter administered by a clinical psychologist and a public speaking behavioral test was performed. In addition, a psychiatrist (K.W.) administered the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al 1998) to exclude other serious psychiatric disorders. Finally, medical examinations were performed.

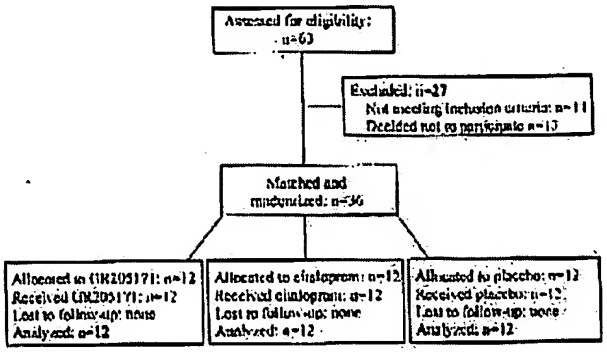

Figure 1. Flow diagram of subject eligibility from screening to statistical analysis.

Main criteria for exclusion were treatment of social anxiety in the past 6 months, current serious or dominant psychiatric disorder other than social phobia (e.g., psychosis, major depressive or bipolar disorder), neurological disorders, somatic disease, chronic use of prescribed medication, abuse of alcohol/narcotics, pregnancy, menopause, left handedness, previous PET examination, and positive family history of cancer.

Approvals were obtained from the Uppsala University Medical Faculty Ethical Review Board, the Uppsala University Isotope Committee, and the Swedish Medical Products Agency. A written informed consent was obtained from all participants.

Study Population

Thirty-six patients (17 men and 19 women; mean age ± SD: 31.6 ± 7.7 years; range 19–48) were included. All participants met the DSM-IV (American Psychiatric Association 1994) criteria for social phobia and exhibited marked public speaking anxiety. Nineteen (52.8%) patients were diagnosed with generalized social phobia and eight qualified for a comorbid diagnosis (three with specific phobia, four with generalized anxiety disorder, and one with both disorders).

Prior to the first PET investigation, patients were matched for severity in triplets based on the Social Phobia Screening Questionnaire (Furmark et al 1999) and also, as far as practically possible, for sex and age. Patients were thereafter randomly allocated to one of three groups: NK1 antagonist, SSRI, or placebo ($n = 12$ per group). Mean age $F(2,33) = .87$, ns, sex ($\chi^2 = .89$, ns), and subtype ($\chi^2 = 1.56$, ns) distributions did not differ significantly across study groups. Patients with comorbid anxiety disorders were distributed equally across the NK1 and SSRI groups (four each). The progress of eligible subjects from screening to analysis is described in Figure 1.

Treatment Procedure

The study was double blind. GlaxoSmithKline (Verona, Italy) supplied the study drugs for a 6-week treatment period. The NK1 group received a daily oral dose of 5 mg GR205171, which started after 14 days of placebo because of limited available safety data on repeated dosing. GR205171 was taken as 4 mL solution made up to 100 mL in orange juice. The SSRI group was treated with 40 mg citalopram (one tablet), starting with 20 mg (half tablet) during the first week. To maintain study blindness, the NK1 and SSRI groups received tablets and solution as dummy

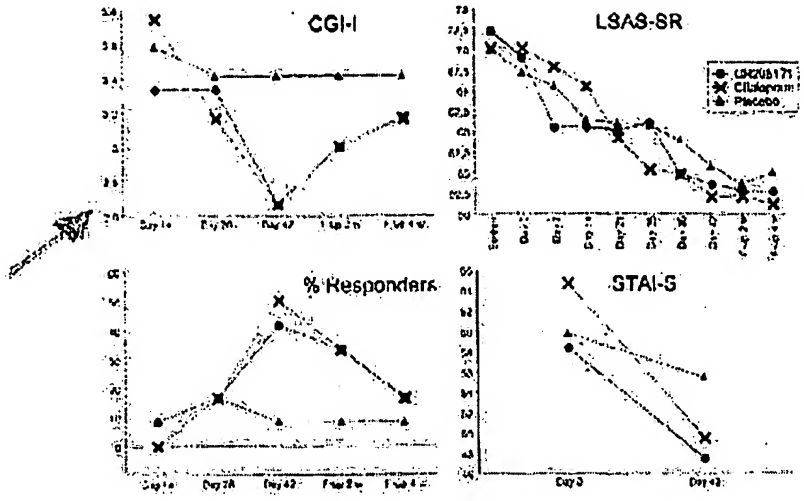

Figure 2. Change scores for primary clinical outcome measures, i.e., the Clinical Global Impression Improvement subscale (top left), percentage of responders over time (bottom left), the Liebowitz Social Anxiety Scale (LSAS-SR, top right) and state anxiety during public speaking challenge measured by the Spielberger State-Trait Anxiety Inventory (STAI-S, bottom right). The treatment started postscreening on day 0, terminated on day 42, and was followed up 2 and 4 weeks later (F-up 2/4 w).

treatments, respectively. The placebo group received dummy treatments matching GR205171 solution and citalopram tablets. All subjects started with half a tablet the first week.

In all groups, the first dose was given immediately after the first PET examination and the final dose was administrated 2 to 4 hours before the second PET assessment on day 42. Subjects did not receive any other form of treatment than the one allocated in the study and no systematic exposure instructions were given.

Patients visited the clinic weekly for assessments of compliance and side effects and to receive new supplies of medication. Vital signs (heart rate, blood pressure) were checked, laboratory safety tests (hematology, biochemistry, and urine analysis) were performed, and self-report questionnaires were administered. Pregnancy tests and electrocardiography were performed twice. Screenings for alcohol and nonallowed drugs were performed at a randomly selected visit.

Follow-up assessments were performed 2 and 4 weeks after the treatment period. Checking of vital signs, safety tests, and questionnaire administration were then repeated. After completion, patients were offered further psychiatric consultation and additional therapy with market drugs.

PET Assessments

Investigations were performed using a 32-ring ECAT EXACT HR+ camera (Siemens/CTI, Knoxville, Tennessee). The camera enables acquisition of 63 contiguous planes of data with a distance of 2.46 mm, resulting in a total axial field of view of 155 mm.

Subjects were positioned in the scanner with the head gently fixated, and a venous catheter for tracer injections was inserted. Patients were instructed to prepare a 2.5-minute speech about a vacation or travel experience about 20 minutes before the initial emission scan. A 10-minute transmission scan was performed using three retractable germanium (^{68}Ge) rotating line sources. The ^{15}O-water tracer, approximately 10 MBq/kg body weight, was thereafter injected intravenously. The emission scan started automatically in three-dimensional (3-D) mode when the bolus reached the brain (50,000 counts/second) and consisted of three 30-second frames.

Immediately following tracer injection, patients were asked to start speaking and continue until they received instructions to stop. The speech was performed in the presence of a silently observing audience of six to eight persons. Patients were instructed to observe the audience. The speech was recorded from close distance with a portable video camera to increase observational anxiety and document verbal performance. Heart rate was recorded simultaneously. Directly after the speech, state anxiety scales (see below) were administered to estimate retrospectively how anxious patients felt during scans.

Emission scans were reconstructed with a filter back projection using an 8-mm Hanning filter, resulting in a spatial resolution of about 5 mm in the field of view. The matrix included 128 × 128 pixels. Data were corrected for photon attenuation, decay, scattered radiation, and random coincidences. After reconstruction, a summation image of the three frames was made to obtain a better statistical reference for realignment and subsequent analyses.

Participants fasted 3 hours and refrained from tobacco, alcohol, and caffeine for 12 hours before PET investigations. The PET procedure was the same after treatment but with altered speech topic.

Clinical Outcome Measures

Primary Outcome Measures. Response rate was determined by the Clinical Global Impression Improvement item (CGI-I) (Zaider et al 2003) administered by a psychiatrist (K.W.) at weeks 2, 4, and 6 and at follow-ups. Patients having a score of 1 or 2 (i.e., very much or much improved) on the CGI-I on day 42 were classified as responders, whereas those having scores of 3 (minimally improved) or higher were considered as nonresponders.

Changes in state anxiety from pretreatment to posttreatment were evaluated using the Spielberger State-Trait Anxiety Inventory (STAI-S) (Spielberger et al 1970), administered after each public speaking challenge. Additional changes in the social phobia symptom profile over the treatment course were evaluated by the self-report version of the Liebowitz Social Anxiety Scale (LSAS-SR) (Baker et al 2002).

Secondary Outcome Measures. The CGI severity subscale (CGI-S) was administered in addition to the global improvement item (CGI-I). Further, patients completed a battery of questionnaires at screening and day 42: Social Phobia Screening Questionnaire (SPSQ) (Furmark et al 1999), the Social Phobia Scale (SPS) and Social Interaction Anxiety Scale (SIAS) (Mattick and Clarke 1998), Global Assessment of Functioning (GAF) self-

Table 1. Temporal Lobe Regions Showing Decreased Within-Group Activation After Treatment of Social Phobia

Group/Brain Region[a]	Coordinate[b] x	y	z	z-Score	Voxel p Value[c]	Cluster p Value[c]
GR205171 (n = 12)						
Left Inferior Temporal Cortex, BA38	−20	−6	−33	4.18	.009	.047
BA28	−20	2	−30	3.09		
Amygdala	−18	−3	−22	2.31		
Citalopram (n = 12)						
Left Inferior Temporal Cortex, BA 20	−24	−8	−38	3.84	.029	.010
BA36	−24	−6	−33	3.51		
BA28	−20	1	−29	2.31		
BA35	−20	−9	−26	2.08		
Amygdala	−26	−3	−22	2.30		
Left Parahippocampal Cortex, BA 27	−14	−31	−4	4.06	.014	ns
Right Superior Temporal Cortex, BA38	36	−1	−10	3.81	.031	.004
BA36	28	−7	−33	3.28		
BA21	42	−2	−10	3.10		
BA13	40	5	−10	2.96		
BA38	30	16	−34	2.30		
BA20	30	−11	−30	2.09		
BA28	28	−11	−30	2.00		
Amygdala	28	−8	−13	2.22		
Placebo (n = 12)					ns	ns
Responders (n = 12)						
Left Inferior Temporal Cortex, BA36[d]	−18	−1	−29	2.63	ns	.040
BA28	−16	−1	−25	2.47		
BA35	−22	−5	−25	2.03		
BA34	−18	3	−22	2.00		
Right Inferior Temporal Cortex, BA36[e]	30	−3	−27	3.52	.075	.022
BA20	40	−21	−28	2.29		
Amygdala	28	−3	−22	2.96		

BA, Brodmann area; NK1, neurokinin-1.

[a]Location of maximum voxel value (presented first) and spatial extention of significant clusters are listed. GR205171 is a NK1-antagonist. Responders = scores 1–2 on the Clinical Global Impression improvement item.

[b]Coordinates in millimeters correspond to the stereotactic atlas of Talairach and Tournoux (1988).

[c]Corrected for multiple comparisons.

[d]Left amygdala implicated at lower threshold (−22 −1 −22; z-score = 1.80).

[e]Right hippocampus implicated at lower threshold (28 −9 −21; z-score = 1.78).

report scale (Bodlund et al 1994), Personal Report on Confidence as a Speaker (PRCS) (Paul 1966), and Sheehan Disability Inventory (SDI) (Leon et al 1992). Heart rate (HR), calculated from the interbeat interval and expressed in beats per minute, was recorded during all public speaking tasks by means of the PSYTAB6 integrated system for psychophysiology (Contact Precision Instruments, London, United Kingdom). Subjects also rated levels of fear and distress, associated with the speaking tasks, on 0 to 100 (minimum to maximum) visual analogue scales (Furmark et al 2002). These secondary measures were included to obtain a more complete clinical picture and for long-term research purposes.

Statistical Analyses

Positron Emission Tomography Data Analyses. Positron emission tomography images were realigned to correct for different positions between scans and normalized to the Montreal Neurological Institute's (MNI) stereotactic template (ICBM 152), using the Statistical Parametric Mapping (SPM99) software (Wellcome Department of Cognitive Neurology, London, United Kingdom). Images were then smoothed using a 12-mm Gaussian kernel. Positron emission tomography data were statistically evaluated using within-group and between-group comparisons defined in SPM99, with rCBF data fitted to the general linear model (Friston et al 1995). Between-group differences were evaluated by group × time interactions in the form of double subtractions, such as $(NK1_{post} - NK1_{pre}) - (Placebo_{post} - Placebo_{pre})$. Differences in global blood flow were corrected for using the proportional scaling method within SPM99. Contrasts generated t-maps, subsequently converted to z-scores, for interpretation. Brain locations are described as xyz coordinates in the Talairach space, obtained by mathematical transformation of the MNI coordinates in SPM99 (www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html). Anatomical localization was supported by searches in the Talairach atlas (Talairach and Tournoux 1988) and the Talairach Daemon (Lancaster et al 2000).

In line with our a priori hypothesis, primary analyses were focused on the medial temporal lobe. A circumscribed search volume for the right and left MTL was created by defining a 26 × 46 × 46 mm box containing 6877 voxels (1 voxel = 2 × 2 × 2 mm) in each hemisphere at the level of the inferior hippocampus. Treatment effects on rCBF were evaluated at the voxel level by examining statistically significant changes ($p < .05$) corrected for multiple comparisons in the defined volume. The spatial extent of voxels exceeding the significance threshold was also examined when motivated by significant cluster p-values (corrected) in the volume of interest. In addition, exploratory whole-brain analyses were performed

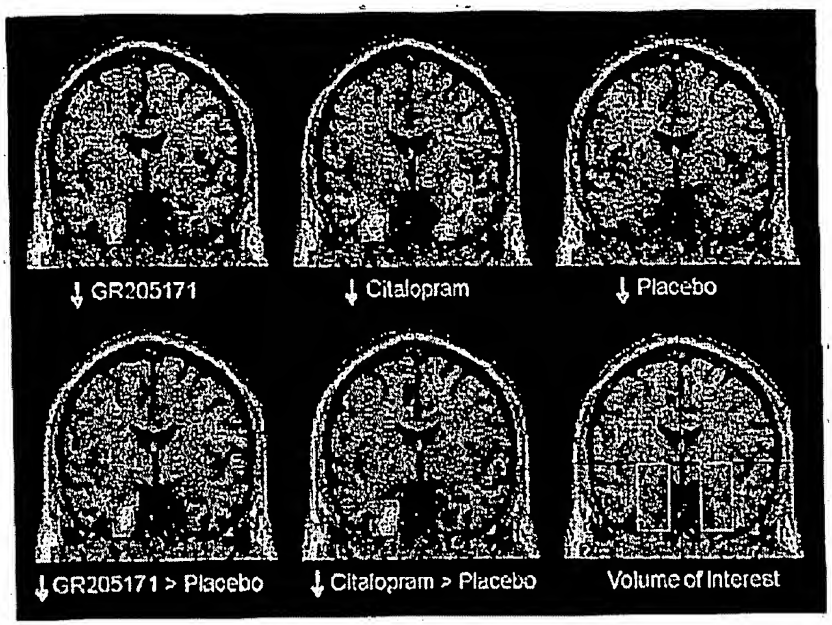

Figure 3. Coronal PET images of patients with social phobia showing clusters of significantly reduced rCBF in the medial temporal lobe during public speaking, after as compared with before treatment, within the NK1 antagonist GR205171 (top left), citalopram (top central), and placebo (top right panel) groups. All groups included 12 subjects each. Between-group comparisons revealed a significantly larger reduction of rCBF in subjects treated with GR205171 ($n = 12$; bottom left) and citalopram ($n = 12$; bottom middle) compared with placebo ($n = 12$). Bottom right panel illustrates the volume of interest used for all hypothesis-driven analyses of rCBF changes in the left and right medial temporal lobe. PET, positron emission tomography; rCBF, regional cerebral blood flow.

evaluating activity changes exceeding $p < .05$, corrected for multiple comparisons.

Clinical Outcome. Data were scanned for violations of normality and heterogeneity of variance and between-group differences at pretreatment were tested by analysis of variance (ANOVA). The distribution of responders/nonresponders, according to the CGI-I on day 42, was evaluated using exact single-cell tests (Bergman and El-Khouri 1987). Planned t tests (paired, two-tailed) were used to detect within-group changes from pretreatment to posttreatment. Between-group differences were tested by pairwise comparisons of the adjusted mean values following analyses of covariance (ANCOVA) with posttreatment score as dependent variable and pretreatment score as covariate in the statistical model. Repeated measurement ANOVA was used to evaluate the LSAS-SR. Analyses were performed using StatView 5.0.1 (SAS Institute Inc., Cary, North Carolina) and Statistica 6.0 (StatSoft Inc, Tulsa, Oklahoma). The alpha-level used was $p < .05$ in all tests.

Verbal Performance. Verbal performance was evaluated by comparing the number of spoken syllables during the first 10 seconds of each videotaped speech, using a repeated measurement ANOVA.

Results

Pretreatment Evaluation

There were no significant differences between groups before treatment on any primary ($.027 < F < 1.38$; $.27 < p < .93$) or secondary ($.22 < F < 3.20$; $.054 < p < .80$) clinical outcome measure.

Primary Clinical Outcome Measures

Response Rate. On day 42 (end of treatment), the numbers of CGI responders were 5 (41.7%) in the NK1 group, 6 (50%) in the citalopram group, and 1 (8.3%) in the placebo group (Figure 2). Eleven nonresponders (NK1/citalopram/placebo = 4/4/3) were "minimally improved" (CGI-I = 3), whereas 13 (NK1/citalopram/placebo = 3/2/8) were categorized as "no change" (CGI-I = 4). Exact single-cell tests showed evidence of a statistically significant association between CGI responders and group ($p = .026$). The two drug groups deteriorated after treatment withdrawal on day 42, whereas placebo subjects did not change.

State Anxiety. Both the NK1 [$t(11) = 3.87$; $p = .0026$] and citalopram [$t(11) = 7.26$, $p < .0001$] groups improved significantly on the STAI-S from pretreatment to posttreatment, whereas the placebo group did not [$t(11) = 1.53$, nsl. A significant effect of group was noted in the ANCOVA of posttreatment scores [$F(2,32) = 4.13$, $p = .025$] and pairwise comparisons showed that both the NK1 ($p = .031$) and citalopram ($p = .013$) groups were significantly more improved than placebo on the STAI-S (Figure 2). Speech ratings were always higher than ratings during a preceding control assessment ($p < .0001$).

Liebowitz Social Anxiety Scale. All groups improved significantly on the LSAS-SR ($2.94 < t < 3.97$; $df = 11$; $.0022 < p < .014$) from screening to day 42 (Figure 2). Repeated measure ANOVAs of the LSAS-SR scores revealed a significant main effect of time [$F(2,33) = 17.0$, $p < .0001$] but no significant effect of group or time × group interaction.

Regional Cerebral Blood Flow

Medial Temporal Lobe Analyses. Within the NK1 and citalopram groups, the rCBF response was significantly lower after treatment in the perirhinal, entorhinal, and parahippocampal cortices, as well as the amygdala. This pattern was bilateral in citalopram subjects but localized mainly to the left

Table 2. Temporal Lobe Regions Showing Decreased Between-Group Activation After Treatment of Social Phobia

Comparison/Brain Region[a]	Coordinate[b] x	y	z	z-Score	Voxel p Value[c]	Cluster p Value[c]
GR205171 vs. Placebo (n = 12/12)						
Left Inferior Temporal Cortex, BA36[d]	−18	−7	−32	4.42	.004	.036
BA28	−20	−11	−30	3.72		
BA35	−18	−11	−25	3.09		
BA34	−16	−7	−22	2.73		
BA30	−22	−6	−32	2.37		
Amygdala	−18	−5	−22	2.46		
Citalopram vs. Placebo (n = 12/12)						
Left Inferior Temporal Cortex, BA36[d]	−20	−7	−32	3.84	.028	.018
BA28	−20	−11	−31	3.46		
BA35	−22	−11	−25	2.80		
Hippocampus	−24	−11	−21	2.32		
Responders vs. No change (n = 12/13)						
Right Parahippocampal Cortex, BA28	28	−1	−25	3.53	.072	.034
BA38	26	5	−25	2.84		
BA28	18	5	−25	2.79		
BA36	16	−1	−27	2.01		
Amygdala	28	−3	−22	3.41		
Hippocampus	30	−24	−9	2.11		

[a] BA, Brodmann area; NK1, neurokinin-1.
[b] Location of maximum voxel value (presented first) and spatial extention of significant clusters are listed. GR205171 is a NK1 antagonist. Responders = scores 1–2 and No change = 4 on the Clinical Global Impression Improvement item.
[c] Coordinates in millimeters correspond to the stereotactic atlas of Talairach and Tournoux (1988).
[d] Corrected for multiple comparisons.
[e] Left amygdala implicated at lower threshold (−24 −5 −22; z-score = 1.81).
[f] Left hippocampus implicated at lower threshold (−24 −11 −21; z-score = 1.75).

hemisphere in the NK1 group. No significant changes were observed in the placebo group (Table 1, Figure 3). Between-group comparisons confirmed that rCBF in the MTL region was significantly more reduced after drug treatment compared with placebo (Table 2, Figure 3). Decreases of rCBF in the hippocampus proper were also noted in these between-group comparisons. The NK1 and citalopram groups did not differ significantly.

Follow-up analyses revealed that responders, irrespective of treatment modality, exhibited significantly lower rCBF after treatment bilaterally in the rhinal and parahippocampal cortices, as well as the amygdala region. A between-group comparison showed that rCBF in the previously noted MTL domain was more reduced in responders (CGI-I ≤ 2) than in patients that did not change (CGI-I = 4), but this pattern was significant only in the right hemisphere (Table 2, Figure 4). In the active drug groups, rCBF alterations were further characterized by comparing subjects that differed in state-anxiety reduction. Subjects were ranked within each of the state anxiety measures (STAI-S, HR, Fear, and Distress) using change scores from pretreatment to posttreatment. The four rankings were summed and a median split of the summed rank was used to define subgroups showing either a large (n = 6) or small (n = 6) anxiety reduction. Both in NK1 and citalopram subjects, the rCBF decrease in the MTL region was significant only in the subgroups exhibiting large anxiety reduction (Table 3, Figure 4).

Whole Brain Analyses. In the NK1 group, rCBF increased significantly in a cluster located in the left occipital cortex (Brodmann area [BA] 17) (-22 -87 -1; z-score 4.08, p = .008). Citalopram subjects exhibited a significant decrease of rCBF in a cluster in the posterior cingulate cortex (BA 31) (-18 -31 38; z-score 4.11, p = .032). In placebo subjects, rCBF increased significantly in the left cerebellum (-2 -76 -13; z-score 4.64, p = .039).

There were no significant effects of group [$F(2,33)$ = 1.11, ns], time [$F(1,33)$ = 1.14, ns], or group × time interaction [$F(2,33)$ = 1.48, ns] with regard to global flow.

Secondary Clinical Outcome Measures

Results on the secondary outcome measures are presented in Table 4. The NK1 group improved significantly on eight (CGI-S, SPSQ, SPS, SIAS, GAF, Fear, Distress, HR), the citalopram group on six (CGI-S, SPSQ, SPS, SIAS, GAF, Distress), and the placebo group on four (CGI-S, SPSQ, SPS, Fear) measures. The PRCS and SDI scales were insensitive to changes in all groups (data not shown). At posttreatment, the ANCOVAs did not reveal significant effects of group on any of the secondary measures [$F(2,32)$ = .02–1.07, ns] and the pairwise comparisons of the adjusted means remained insignificant.

Verbal Performance

No significant effects of group [$F(2,30)$ = .59, ns], time [$F(1,30)$ = .06, ns], or group × time interaction [$F(2,30)$ = .55, ns] were noted regarding the number of spoken syllables.

Adverse Events

There were 26, 43, and 23 drug-related adverse events in the NK1, citalopram, and placebo groups, respectively, the most common being headache, tiredness, insomnia, nausea, irritability, and somnolence. Events were generally mild or moderate and all were resolved. No subject expressed a wish to discontinue the study.

Discussion

This study explored changes in rCBF following short-term treatment with the NK1 antagonist GR205171, compared with

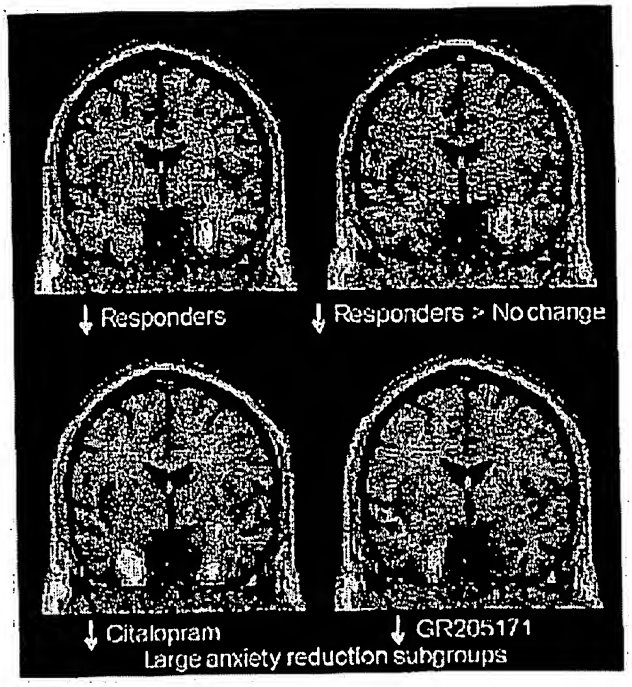

Figure 4. Follow up PET analyses. Coronal images of significantly reduced rCBF during public speaking in the medial temporal lobe after treatment in responders on the Clinical Global Impression improvement scale (*n* = 12; top left) and in responders compared with subjects that did not change on this scale (*n* = 13; top right). Further analyses showed significantly decreased rCBF in patients that exhibited the largest reduction of state anxiety from pretreatment to posttreatment within the citalopram (bottom left) and the NK1 antagonist GR205171 (bottom right) groups. No significant rCBF changes were observed in the remaining subjects exhibiting a smaller anxiety reduction (not illustrated). PET, positron emission tomography; rCBF, regional cerebral blood flow.

citalopram and placebo, in patients with social phobia. Posttreatment assessments (day 42) suggested that the NK1 and SSRI treatments reduced the neural response to public speaking in the MTL, including the perirhinal, entorhinal, and parahippocampal cortices, as well as the amygdala. This effect was also observed in responders as defined by the CGI-I, regardless of treatment modality, but not in the placebo group or in subjects that did not change clinically. Between-group comparisons confirmed larger rCBF decrement in the MTL, including the hippocampus proper, in both active drug groups relative to placebo and in responders relative to subjects that did not change. Within the NK1 and SSRI groups, the rCBF decrease was mediated predominantly by subjects showing a large reduction of public speaking state anxiety from pretreatment to posttreatment.

The clinician's ratings (CGI-I) and differential reduction of state anxiety during the public speaking task (STAI-S) suggested that both the NK1 antagonist and the SSRI were superior to placebo. In both drug groups, symptoms deteriorated 2 and 4 weeks after treatment withdrawal, supporting a pharmacological effect. Significant within-group improvement was observed on the majority of secondary measures after active drug treatment, although the placebo group also improved on some scales. On all measures, the anxiolytic effect of the NK1 antagonist was similar to that of citalopram, even though it was administered for a shorter period, i.e. 4 as compared with 6 weeks. Verbal performance, indexed by number of spoken syllables, was similar before and after treatment in all groups. This pattern supports that the observed rCBF alterations were specifically related to social anxiety reduction following active drug treatment.

The present results are in good agreement with our previous study of social phobia in which symptom improvement with 9 weeks of either citalopram or cognitive-behavioral therapy was associated with reduced rCBF during public speaking in the MTL region (Furmark et al 2002). Both studies strongly indicate that down-regulation of MTL neural activity is an important mechanism in the alleviation of social anxiety. The present findings are also congruent with previous neuroimaging studies in social phobia that have suggested a role for the MTL, or the amygdala, in situationally elicited (Tillfors et al 2001) and anticipatory (Lorberbaum et al 2004; Tillfors et al 2002) anxiety, aversive conditioning (Schneider et al 1999), and in the perception of harsh (Stein et al 2002) and neutral (Birbaumer et al 1998; Veit et al 2002) facial expressions. Taken together, these imaging studies suggest exaggerated responsiveness of the MTL in social phobia and that effective treatment attenuates anxiety-related neural activation in this region. In depressed patients, some investigations have noted that antidepressant drug treatment reduces resting state amygdala hypermetabolism (Drevets et al 2002) and exaggerated amygdala responses to masked fearful faces (Sheline et al 2001). This could imply that the amygdala is a general target for treatments of disorders characterized by negative affect.

The amygdala has long been implicated in the acquisition and expression of fear-related behavior (LeDoux 1996, 2000). It may be particularly important in attention and vigilance, or when the meaning of stimuli is detected, in aversive or ambiguous contexts (Davis and Whalen 2001). The amygdala also has a role in social perception and judgment (Adolphs 2003), which may have specific relevance for social phobia (Amaral 2002). Increased hippocampal activation in frightening situations might be attributed to cognitive processes or contextual evaluation, whereas the surrounding perirhinal, entorhinal, and parahippocampal cortices could be an important transit area for sensory and/or memory information into the subcortical structures (LeDoux 1996). The amygdala is not necessarily the main site of action in the alleviation of anxiety. Neuroimaging studies of anxiety pathways often report conjoint activation of a larger MTL region, comprising both subcortical and cortical areas, rather than an isolated activation of the amygdala (Furmark et al 2002; Stein et al 2002; Tillfors et al 2001, 2002). It is plausible that MTL structures function collectively as an affect-sensitive network that is triggered by threatening stimulation. Selective serotonin reuptake inhibitors might attenuate this network either directly or indirectly, e.g., by balancing median raphe nucleus firing or through interactions with other transmitter systems and pathways (Grove et al 1997). Enhanced serotonergic tone after SSRI treatment could have inhibitory influences on thalamic and cortical inputs to the amygdala (Gorman et al 2000; Stein and Stahl 2000) and presumably other MTL areas. However, the role of serotonin in anxiety is complex since it may have opposite effects in different neural pathways (Graeff 2002). Serotonergic modulation of anxiety may involve both presynaptic and postsynaptic processes and numerous receptor subtypes (Kent et al 2002b; Sandford et al 2000). Studies of serotonin transporter functions could also help to unravel the

Table 3. Temporal Lobe Regions Exhibiting Decreased Activation After Treatment of Social Phobia in Subjects that Showed Either Large or Small Reduction in Public Speaking State Anxiety

Group/Brain Region[a]	Coordinate[b] x	y	z	z-score	Voxel-p Value[c]	Cluster p Value[c]
GR205171: Large Reduction ($n = 6$)						
Left Inferior Temporal Cortex, BA36	−22	−2	−34	3.22	ns	.049
BA38	−22	4	−34	2.89		
Amygdala	−18	−3	−22	2.10		
Citalopram: Large Reduction ($n = 6$)						
Left Inferior Temporal Cortex, BA28	−30	1	−29	4.13	.011	.002
BA36	−24	−4	−33	3.01		
BA20	−26	−4	−35	3.77		
BA38	−24	2	−35	3.04		
Amygdala	−26	−1	−18	2.47		
Right Inferior Temporal Cortex, BA20[d]	28	−10	−37	3.60	.060	.001
BA36	30	−6	−33	3.39		
BA38	24	2	−35	2.12		
Amygdala	28	−5	−13	2.48		

BA, Brodmann area; NK1, neurokinin-1.

[a]Location of maximum voxel value (presented first) and spatial extention of significant clusters are listed. GR205171 is a NK1-antagonist. No significant changes were noted in the small reduction subgroups.

[b]Coordinates in millimeters correspond to the stereotactic atlas of Talairach and Tournoux (1988).

[c]Corrected for multiple comparisons.

[d]Right hippocampus implicated at lower threshold (30, −11, −20; z-score = 1.77).

mechanisms whereby the SSRIs act in social phobia (Kent et al 2002a) and other disorders.

Consistent with data previously reported by Kramer et al (1998) in depressed patients, the NK1 antagonist and SSRI reduced anxiety to a similar extent. The current PET data suggest that attenuation of MTL neural activity could be an important anxiolytic mechanism also in NK1-targeted pharmacotherapy. The anxiolytic effect of NK1 antagonists like GR205171 may be attributed to reduced SP neurotransmission or subsequently lowered levels of central SP, as suggested by animal studies (Häsenohrl et al 2000), resulting in a net inhibition of MTL neural activity. However, this could also be the result of an interaction with other neurotransmitters. For instance, SP may coexist in serotonergic neurons, thereby modifying their release and effects (Häsenohrl et al 2000). Drugs that act on serotonin neurotransmission can reduce levels of central SP, e.g., in the amygdala (Shirayama et al 1996). Animal studies indicate that the anxiolytic effect following genetic disruption of the NK1 receptor is paralleled by increased firing of serotonergic neurons in the dorsal raphe nucleus and desensitization of inhibitory serotonin-1A autoreceptors (Santarelli et al 2001). Recently, it was demonstrated that SP also might interact with the gamma-aminobutyric acid (GABA)ergic system (Ribeiro and De Lima 2002).

Besides the MTL, a few other regions showed altered activation with treatment. In the NK1 group, rCBF increased significantly in the left occipital cortex. Speculatively, this could be related to improved visual attention which otherwise appears to be shifted away from potentially threatening environmental cues in social phobia (Chen et al 2002). Consistently, a recent PET study noted deactivation of the visual cortex during symptom provocation in male patients with generalized social phobia (Van Ameringen et al 2004). Citalopram subjects showed rCBF diminution in the posterior cingulate cortex. This region, in particular the retrosplenial cortex, has been linked to episodic memory retrieval and is frequently activated in imaging studies of emotional processing (Maddock 1999). Activation of the left cerebellum was noted in placebo subjects, possibly reflecting alterations in motor activity or cognitive processes (Allen et al 1997).

The present study cannot determine whether true normalization of MTL activity occurred, because pretreatment rCBF values were not compared with nonfearful control subjects. We previously observed that the amygdalohippocampal response to a stressful speaking task was enhanced in untreated patients with social phobia relative to nonanxious control subjects (Tillfors et al 2001). Thus, treatment may normalize preexisting abnormalities in the MTL. However, at baseline, phobic subjects and control subjects differed also in widespread cortical regions (Tillfors et al 2001) that remained unaffected in the present but also in our previous (Furmark et al 2002) treatment study. Congruently, imaging studies of major depression suggest that treatments involve both normalization and other adaptive metabolic changes in the brain (Mayberg et al 2000).

Among the limitations, it should be noted that although a sample size of 12 subjects per group yields sufficient power to demonstrate rCBF changes (Andreasen et al 1996), larger sample sizes are generally required to verify differences between active treatment and placebo on behavioral measures. A clear differential response between drug treatment and placebo was found only on the STAI-S and CGI-I, whereas robust between-group differences were not observed on the LSAS-SR or the secondary measures. This could also be due to short treatment periods and limited scale sensitivity. Measures of brain activity may have greater sensitivity than behavioral measures when evaluating emotional reactions (Harari et al 2002).

In this study, social anxiety reactions may differ from naturalistic settings, and the most severe cases of social phobia were perhaps not willing to participate, which could restrict the external validity. Another limitation is the lack of a control condition without which it cannot be ruled out that rCBF changes reflect nonspecific drug effects on cerebral

Table 4. Mean (SD) and Paired t Values for Secondary Clinical Outcome Measures Before and After Treatment

Measure		GR205171	Citalopram	Placebo
CGI-S	pre	4.8 (.9)	4.7 (1.1)	4.8 (.8)
	post	3.6 (.9)	3.6 (1.7)	4.1 (1.2)
	t(11)	4.84[d]	5.61[d]	3.45[b]
SPSQ	pre	33.6 (6.9)	28.7 (9.2)	32.3 (7.2)
	post	25.1 (6.3)	22.0 (9.4)	24.8 (7.3)
	t(11)	4.51[d]	3.43[b]	3.85[c]
SPS	pre	34.0 (10.5)	31.3 (13.5)	37.1 (17.2)
	post	22.8 (8.5)	22.5 (11.8)	29.3 (15.6)
	t(11)	5.91[d]	4.26[c]	2.37[a]
SIAS	pre	50.5 (11.8)	44.8 (16.0)	50.0 (11.4)
	post	38.9 (11.9)	35.3 (16.2)	42.2 (10.9)
	t(11)	9.21[d]	4.94[d]	2.1
GAF	pre	74.5 (12.1)	72.7 (12.7)	76.3 (11.5)
	post	66.2 (8.6)	81.5 (10.3)	82.9 (8.6)
	t(11)	3.76[c]	3.13[b]	2.1
Fear	pre	57.1 (27.4)	62.7 (28.8)	55.9 (26.9)
	post	33.9 (19.4)	50.0 (26.4)	39.4 (35.7)
	t(11)	4.15[c]	1.62	2.70[a]
Distress	pre	69.3 (22.5)	68.9 (25.4)	62.5 (28.8)
	post	46.7 (19.3)	52.1 (27.3)	52.1 (31.0)
	t(11)	3.36[b]	2.92[a]	2.05
HR	pre	82.5 (10.7)	85.2 (19.7)	87.9 (13.5)
	post	76.0 (18.7)	79.5 (15.7)	83.7 (15.2)
	t(11)	2.34[a]	1.91	1.42

GR205171 is a Neurokinin-1 antagonist.
CGI-S, Clinical Global Impression severity scale; SPSQ, Social Phobia Screening Questionnaire; SPS, Social Phobia Scale; SIAS, Social Interaction Anxiety Scale; GAF, Global Assessment of Functioning self-report; HR, Heart rate.
[a] $p < .05$.
[b] $p < .01$.
[c] $p < .005$.
[d] $p < .001$.

vascular functions. However, in both drug groups, the rCBF reduction was significant only in subjects showing large state anxiety reduction and not in those showing a small anxiety reduction, in spite of similar drug intake. This suggests that nonspecific vascular effects are unlikely to explain the rCBF changes observed in MTL during public speaking. Further, nonspecific vascular effects are not compatible with the fact that global blood flow did not change with treatment. It has previously been reported that chronic treatment with fluoxetine does not affect regional or global cerebral blood flow in healthy volunteers (Bonne et al 1999).

The trial reported here was experimental, and in clinical practice, longer treatment periods are generally required to obtain robust and enduring therapeutic effects. For the SSRIs, the anxiolytic effect is typically seen after 2 to 6 weeks (Bandelow and Stein 2004), but additional improvement may continue over a considerable time span (Blomhoff et al 2001). Future imaging studies could use more extended treatment periods and more assessment points and relate anxiety reduction not only to changes in rCBF but also to neurotransmitter/receptor functions such as serotonin synthesis and NK1 receptor occupancy. Neuroimaging techniques could also be used to study dose-response relationships, drug-psychotherapy combinations, and genetic influences on treatment outcome and symptom severity (Furmark et al 2004).

In conclusion, social anxiety was significantly alleviated after short-term treatment with either the NK1 receptor antagonist GR205171 or citalopram. Both drugs were superior to placebo in terms of response rate and reduction of public speaking state anxiety. Neurokinin-1 receptor blockade, as well as serotonin reuptake inhibition, was associated with reduced neural activity in the MTL, which has been ascribed a crucial role in the regulation of fear and anxiety.

This research was funded by GlaxoSmithKline, with additional support from the Swedish Research Council (MF and TF), the Bank of Sweden Tercentenary Foundation (MF), and the Swedish Brain Foundation (TF).

We wish to express our sincere gratitude to Caroline Blatz, Paolo Fina, Caroline Hyldén-Linnander, Sarah Job, Annika Larsson, Petri Laukka, Susanna Lewenhaupt, Hanna Lundholm, and Peder Palmstierna for invaluable assistance and to the staffs of Uppsala Imanet and Quintiles for providing excellent research conditions.

Adolphs R (2003): Cognitive neuroscience of human social behaviour. *Nat Rev Neurosci* 4:165–178.

Aguiar MS, Brandao ML (1996): Effects of microinjections of the neuropeptide substance P in the dorsal periaqueductal gray on the behaviour of rats in the plus-maze test. *Physiol Behav* 60:1183–1186.

Allen G, Buxton RB, Wong EC, Courchesne E (1997): Attentional activation of the cerebellum independent of motor involvement. *Science* 275:1940–1943.

Amaral DG (2002): The primate amygdala and the neurobiology of social behavior: Implications for understanding social anxiety. *Biol Psychiatry* 51:11–17.

American Psychiatric Association (1994): *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* Washington, DC: American Psychiatric Press.

Ameringen MV, Mancini C, Farvolden P, Oakman J (2000): Drugs in development for social anxiety disorder: More to social anxiety than meets the SSRI. *Expert Opin Investig Drugs* 9:2215–2231.

Andreasen NC, Arndt S, Cizadlo T, O'Leary DS, Watkins GL, Ponto LL, et al (1996): Sample size and statistical power in [15O]H2O studies of human cognition. *J Cereb Blood Flow Metab* 16:004–016.

Baker SL, Heinrichs N, Kim HJ, Hofmann SG (2002): The Liebowitz social anxiety scale as a self-report instrument: A preliminary psychometric analysis. *Behav Res Ther* 40:701–715.

Bandelow B, Stein DJ (2004): Pharmacotherapy of social anxiety disorder. In: Bandelow B, Stein DJ, editors. *Social Anxiety Disorder.* New York: Marcel Dekker 267–297.

Bergman LR, El-Khouri BM (1987): EXACON–a Fortran 77 program for the exact analysis of single cells in a contingency table. *Educ Psychol Meas* 47:155–161.

Birbaumer N, Grodd W, Diedrich O, Klose U, Erh M, Lotze M, et al (1998): fMRI reveals amygdala activation to human faces in social phobics. *Neuroreport* 9:1223–1226.

Blomhoff S, Haug TT, Hellstrom K, Holme I, Humble M, Madsbu HP, et al (2001): Randomised controlled general practice trial of sertraline, exposure therapy and combined treatment in generalised social phobia. *Br J Psychiatry* 179:23–30.

Bodlund O, Kullgren G, Ekselius L, Lindström E, von Knorring L (1994): Axis V-Global assessment of functioning: Evaluation of a self-report version. *Acta Psychiatr Scand* 90:342–347.

Bonne O, Krausz Y, Ahron Y, Gelfin Y, Chisin R, Lerer B (1999): Clinical doses of fluoxetine and cerebral blood flow in healthy volunteers. *Psychopharmacology (Berl)* 143:24–28.

Brodin E, Rosen A, Schott E, Brodin K (1994): Effects of sequential removal of rats from a group cage, and of individual housing of rats, on substance P, cholecystokinin and somatostatin levels in the periaqueductal grey and limbic regions. *Neuropeptides* 26:253–260.

Chen YP, Ehlers A, Clark DM, Mansell W (2002): Patients with generalized social phobia direct their attention away from faces. *Behav Res Ther* 40:677–687.

Davidson RJ, Jackson DC, Kalin NH (2000): Emotion, plasticity, context, and regulation: Perspectives from affective neuroscience. *Psychol Bull* 126:890–909.

Davis M, Whalen PJ (2001): The amygdala: Vigilance and emotion. *Mol Psychiatry* 6:13–34.

Drevets WC, Rogers W, Raichle ME (2002): Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. *Eur Neuropsychopharmacol* 12:527–544.

File SE (1997): Anxiolytic action of a neurokinin1 receptor antagonist in the social interaction test. *Pharmacol Biochem Behav* 58:747–752.

File SE (2000): NKP608, an NK1 receptor antagonist, has an anxiolytic action in the social interaction test in rats. *Psychopharmacology (Berl)* 152:105–109.

First MB, Gibbon M, Spitzer RL, Williams JBW (1998): *SCID-I: Interview Protocol* (Swedish). Stockholm: Pilgrim Press.

Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith CD, Frackowiak RSJ (1995): Statistical parametric maps in functional imaging: A general linear approach. *Hum Brain Mapp* 2:189–210.

Furmark T (2002): Social phobia: Overview of community surveys. *Acta Psychiatr Scand* 105:84–93.

Furmark T, Tillfors M, Everz P-O, Marteinsdottir I, Gefvert O, Fredrikson M (1999): Social phobia in the general population: Prevalence and sociodemographic profile. *Soc Psychiatry Psychiatr Epidemiol* 34:416–424.

Furmark T, Tillfors M, Garpenstrand H, Marteinsdottir I, Langstrom B, Oreland L, et al (2004): Serotonin transporter polymorphism related to amygdala excitability and symptom severity in patients with social phobia. *Neurosci Lett* 362:189–192.

Furmark T, Tillfors M, Marteinsdottir I, Fischer H, Pissiota A, Langstrom B, et al (2002): Common changes in cerebral blood flow in patients with social phobia treated with citalopram or cognitive-behavioral therapy. *Arch Gen Psychiatry* 59:425–433.

Gardner CJ, Armour DR, Beattie DT, Gale ID, Hawcock AB, Kilpatrick GJ et al (1996): GR205171: A novel antagonist with high affinity for the tachykinin NK1 receptor, and potent broad-spectrum anti-emetic activity. *Regul Pept* 65:45–53.

Gorman JM, Kent JM (1999): SSRIs and SMRIs: Broad spectrum of efficacy beyond major depression. *J Clin Psychiatry* 60(suppl 4):33–38.

Gorman JM, Kent JM, Sullivan GM, Coplan JD (2000): Neuroanatomical hypothesis of panic disorder, revised. *Am J Psychiatry* 157:493–505.

Graeff FG (2002): On serotonin and experimental anxiety. *Psychopharmacology (Berl)* 163:467–476.

Gray JA, McNaughton N (1996): The neuropsychology of anxiety: Reprise. *Nebr Symp Motiv* 43:61–134.

Griebel G (1999): Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders? *Pharmacol Ther* 82:1–61.

Grove G, Coplan JD, Hollander E (1997): The neuroanatomy of 5-HT dysregulation and panic disorder. *J Neuropsychiatry Clin Neurosci* 9:198–207.

Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, et al (2002): Serotonin transporter genetic variation and the response of the human amygdala. *Science* 297:400–403.

Hasenohrl RU, Souza-Silva MA, Nikulasa S, Tomaz C, Brandao ML, Schwarting RK, et al (2000): Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. *Neuropeptides* 34:272–280.

Kent JM, Coplan JD, Lombardo I, Hwang DR, Huang Y, Mawlawi O, et al (2002a): Occupancy of brain serotonin transporters during treatment with paroxetine in patients with social phobia: A positron emission tomography study with [(11)C]McN 5652. *Psychopharmacology (Berl)* 164:141–148.

Kent JM, Mathew SJ, Gorman JM (2002b): Molecular targets in the treatment of anxiety. *Biol Psychiatry* 52:1008–1030.

Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, et al (1998): Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* 281:1640–1645.

Kramer MS, Winokur A, Kelsey J, Preskorn SH, Rothschild AJ, Snavely D, et al (2004): Demonstration of the efficacy and safety of a novel substance P (NK1) receptor antagonist in major depression. *Neuropsychopharmacology* 29:385–392.

Krase W, Koch M, Schnitzler HU (1994): Substance P is involved in the sensitization of the acoustic startle response by footshocks in rats. *Behav Brain Res* 63:81–88.

Lancaster JL, Woldorff MG, Parsons LM, Liotti M, Freitas CS, Rainey L, et al (2000): Automated Talairach atlas labels for functional brain mapping. *Hum Brain Mapp* 10:120–131.

LeDoux JE (1996): *The Emotional Brain: The Mysterious Underpinnings of Emotional Life*. New York: Simon & Schuster.

LeDoux JE (2000): Emotion circuits in the brain. *Annu Rev Neurosci* 23:155–184.

Leon AC, Shear MK, Portera L, Klerman GL (1992): Assessing impairment in patients with panic disorder: The Sheehan Disability Scale. *Soc Psychiatry Psychiatr Epidemiol* 27:78–82.

Lotherbaum JP, Kose S, Johnson MR, Arana GW, Sullivan LK, Hamner MB, et al (2004): Neural correlates of speech anticipatory anxiety in generalized social phobia. *Neuroreport* 15:2701–2705.

Maddock RJ (1999): The retrosplenial cortex and emotion: New insights from functional neuroimaging of the human brain. *Trends Neurosci* 22:310–316.

Mattick RP, Clarke JC (1998): Development and validation of measures of social phobia scrutiny fear and social interaction anxiety. *Behav Res Ther* 36:455–470.

Mayberg HS, Brannan SK, Tekell JL, Silva JA, Mahurin RK, McGinnis S, et al (2000): Regional metabolic effects of fluoxetine in major depression: Serial changes and relationship to clinical response. *Biol Psychiatry* 48: 830–843.

McLean S, Ganguly AH, Seeger TF, Bryce DK, Pratt KG, Reynolds LS, et al (1991): Activity and distribution of binding sites in brain of a nonpeptide substance P (NK1) receptor antagonist. *Science* 251:437–439.

Paul G (1966): *Insight vs. Desensitization in Psychotherapy*. Stanford, CA: Stanford University Press.

Ribeiro RL, De Lima TCM (2002): Participation of $GABA_A$ receptors in the modulation of experimental anxiety by tachykinin agonists and antagonists in mice. *Prog Neuropsychopharmacol Biol Psychiatry* 26:861–869.

Rupniak NM, Carlson EJ, Shepheard S, Bentley G, Williams AR, Hill A, et al (2003): Comparison of the functional blockade of rat substance P (NK1) receptors by GR205171, RP67580, SR140333 and NKP-608. *Neuropharmacology* 45:231–241.

Rupniak NM, Kramer MS (1999): Discovery of the antidepressant and antiemetic efficacy of substance P receptor (NK1) antagonists. *Trends Pharmacol Sci* 20:485–490.

Sandford JJ, Argyropoulos SV, Nutt DJ (2000): The psychobiology of anxiolytic drugs. Part 1: Basic neurobiology. *Pharmacol Ther* 88:197–212.

Santarelli L, Gobbi G, Debs PC, Sibille ET, Blier P, Hen R, et al (2001): Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proc Natl Acad Sci U S A* 98:1912–1917.

Schneider F, Weiss U, Kessler C, Müller-Gärtner H-W, Posse S, Salloum JB, et al (1999): Subcortical correlates of differential classical conditioning of aversive emotional reactions in social phobia. *Biol Psychiatry* 45:863–871.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al (1998): The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 59(suppl 20):22–33.

Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA (2001): Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: An fMRI study. *Biol Psychiatry* 50:651–658.

Shirayama Y, Mitsushio H, Takashima M, Ichikawa H, Takahashi K (1996): Reduction of substance P after chronic antidepressants treatment in the striatum, substantia nigra and amygdala of the rat. *Brain Res* 739:70–78.

Spielberger CD, Gorsuch RL, Lushene RE (1970): *Manual for the State-Trait-Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Stein DJ, Stahl S (2000): Serotonin and anxiety: Current models. *Int Clin Psychopharmacol* 15(suppl 2):S1–S6.

Stein MB, Goldin PR, Sareen J, Zorrilla LT, Brown GG (2002): Increased amygdala activation to angry and contemptuous faces in generalized social phobia. *Arch Gen Psychiatry* 59:1027–1034.

Talairach J, Tournoux P (1988): *Co-Planar Stereotaxic Atlas of the Human Brain*. Stuttgart, Germany: Georg Thieme Verlag.

Telxeira RM, Santos AR, Ribeiro SJ, Calixto JB, Rae GA, Da Lima TC (1996): Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze behavior in mice. *Eur J Pharmacol* 311:7–14.

Tillfors M, Furmark T, Marteinsdottir I, Fischer H, Pissiota A, Langström B, et al (2001): Cerebral blood flow in subjects with social phobia during stressful speaking tasks: A PET study. *Am J Psychiatry* 158:1220–1226.

Tillfors M, Furmark T, Marteinsdottir I, Fredrikson M (2002): Cerebral blood flow during anticipation of public speaking in social phobia: A PET study. *Biol Psychiatry* 52:1113–1119.

Van Ameringen M, Mancini C, Oakman JM, Farvolden P (1999): Selective serotonin reuptake inhibitors in the treatment of social phobia. The emerging gold standard. *CNS Drugs* 11:307–315.

Van Ameringen M, Mancini C, Szechtman H, Nahmias C, Oakman JM, Hall GB, et al (2001): A PET provocation study of generalized social phobia. *Psychiatry Res* 132:13–18.

van der Linden G, van Heerden B, Warwick J, Wessels C, van Kradenburg J, Zungu-Dirwayi N, et al (2000): Functional brain imaging and pharmacotherapy in social phobia: Single photon emission computed tomography before and after treatment with the selective serotonin reuptake inhibitor citalopram. *Prog Neuropsychopharmacol Biol Psychiatry* 24:419–438.

Varty GB, Cohen-Williams MF, Morgan CA, Pylak U, Duffy RA, Lachowicz JE, et al (2002): The gerbil elevated plus-maze II: Anxiolytic-like effects of selective neurokinin NK1 receptor antagonists. *Neuropsychopharmacology* 27:371–379.

Veit R, Flor H, Erb M, Hermann C, Lotze M, Grodd W, et al (2002): Brain circuits involved in emotional learning in antisocial behavior and social phobia in humans. *Neurosci Lett* 328:233–236.

Wittchen HU, Fuetsch M, Sonntag H, Muller N, Liebowitz M (2000): Disability and quality of life in pure and comorbid social phobia. Findings from a controlled study. *Eur Psychiatry* 15:46–58.

Yonkers KA, Dyck IR, Keller MB (2001): An eight-year longitudinal comparison of clinical course and characteristics of social phobia among men and women. *Psychiatr Serv* 52:637–643.

Zaider TI, Heimberg RG, Fresco DM, Schneier FR, Liebowitz MR (2003): Evaluation of the clinical global impression scale among individuals with social anxiety disorder. *Psychol Med* 33:611–622.

Zamuner S, Gomeni R, Bye A (2002): Estimate the time-varying brain receptor occupancy in PET imaging experiments using non-linear fixed and mixed effect modeling approach. *Nucl Med Biol* 29:115–123.

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Patacchini, R. and Maggi, C.A. Tachykinin NK-1 receptor antagonists. in: Handbook of Experimental Pharmacology, Pharmacology of Tachykinins. P.Holzer Ed. Springer-Verlag, Heidelberg, Germany. 2004

Tachykinin NK_1 Receptor Antagonists

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Abstract This chapter is focused on the pharmacology of most relevant tachykinin NK_1 receptor-selective compounds, with emphasis on the progress of knowledge made possible by their use and on the therapeutic perspectives of these drugs. The first peptide antagonists of SP, synthesized about 20 years ago, were hampered by poor selectivity for NK_1 receptors and other serious side effects. Since then the number of new compounds, peptidic at first and nonpeptidic later, being endowed with increasing potency and selectivity toward the NK_1 receptor, has been growing continuously. A milestone in the field has been the introduction in 1991 of the first nonpeptide compound, CP96345, by Pfizer. CP96345 was instrumental, along with RP 67580 from Rhone-Poulenc, for recognition of the existence of species-related heterogeneity of NK_1 receptors. CP96345 has been used in a plenty of preclinical studies aiming at investigating

the pathophysiological role of the NK_1 receptor, demonstrating the involvement of this receptor in pain perception and inflammation. The following compounds introduced in the field have been deprived of the undesired ion channel-blocking activity accompanying CP96345 and related compounds. By their use, the involvement of the NK_1 receptor in emesis and in mediating affective disorders such as depression and anxiety has been proven. Antiemetic and antidepressive activity in humans has been reported first by CP122721 from Pfizer and by L754030 (MK869) from Merck, respectively. In contrast, disappointing results have been obtained with tachykinin NK_1 antagonists tested as analgesics in patients affected by osteoarthritis, neuropathic pain, dental pain and migraine, for reasons that are still not fully understood. Tachykinin NK_1 receptor-selective antagonists are expected to provide therapeutic effects in peripheral inflammatory diseases such as asthma and inflammatory bowel disease. However, the first trials with these compounds in asthmatic patients have been negative.

Keywords Tachykinins · Tachykinin NK_1 receptor · Tachykinin NK_1 receptor antagonists

1 Introduction

The appearance of the first peptidic antagonists of substance P (SP), at the beginning of the 1980s, triggered intense research programs undertaken by many pharmaceutical companies and which aimed at developing more potent and selective compounds to be used as drugs for treatment of painful/inflammatory conditions in which tachykinins were suspected to play a role. Since then, many potent antagonists of the NK_1 receptor have been developed, being devoid of the numerous drawbacks that characterized the earlier peptide and nonpeptide compounds. The very intense use of these tools in preclinical studies has enabled us to understand the contribution made by tachykinins acting via NK_1 NK_2 and NK_3 receptors to pathophysiological conditions in mammals. As a result of these efforts, several tachykinin NK_1 receptor antagonists are currently under clinical investigation for treatment of pain of different origin, inflammatory bowel disease (IBD), emesis, anxiety and depression. This chapter is focused on the pharmacology of the most relevant tachykinin NK_1 receptor-selective compounds, with emphasis on the progress of knowledge made possible by their use in the tachykinin field, and with appropriate hints at the therapeutic perspectives of these compounds.

2 The Target Receptor

The NK_1 receptor is widely distributed throughout the central and peripheral nervous system of mammals, and was originally defined as the mediator of the biological activities encoded by the C-terminal sequence of tachykinins for

which SP is a more potent agonist than neurokinin A (NKA) or neurokinin B (NKB) (for reviews see Regoli et al. 1989; Guard and Watson 1991; Mussap et al. 1993; Maggi et al. 1993b; Maggi 1995). This concept remained unchanged until Schwartz and coworkers (Hastrup and Schwartz 1996) provided evidence, obtained by radioligand binding and functional experiments, that both NKA and NKB are as potent as SP at stimulating the tachykinin NK_1 receptor (see Sect. 2.1.2): this conclusion has recently been confirmed by in vivo experiments showing NKA to be a potent stimulator of salivary secretion in rats (Bremer et al. 2001). As reviewed elsewhere (Regoli et al. 1994; Maggi 1995; Quartara and Maggi 1997), the tachykinin NK_1 receptor has been cloned from several species, including man. It is worth mentioning that until now only one copy of the corresponding gene has been isolated in man and all other species examined. The NK_1 receptor belongs to the superfamily of rhodopsin-like G-protein-coupled receptors with seven transmembrane spanning segments. Stimulation of the NK_1 receptor leads to generation of at least three different second messenger pathways: phosphatidyl inositol breakdown (leading to elevation of intracellular calcium); arachidonic acid mobilization (leading to prostanoid generation) and cAMP accumulation (for a review see Quartara and Maggi 1997).

2.1 Heterogeneity of NK_1 Receptors Revealed by Selective Antagonists

2.1.1 Species-Dependent Heterogeneity of the NK_1 Receptor

The introduction of the first potent and selective nonpeptide antagonists of the NK_1 receptor, namely CP96345 and RP67580 (see Sects. 4.1 and 4.2), gave a boost to the research aimed at investigating the role of the NK_1 receptor as mediator of tachykinin-induced effects in mammals. From the many studies which were undertaken, a striking pharmacological heterogeneity among NK_1 receptors belonging to different species emerged. For example, CP96345 was found to be 30–100-fold more potent in displacing radiolabeled SP from human, guinea pig, bovine, hamster, gerbil and rabbit than from rat or mouse NK_1 receptors (Gitter et al. 1991; Beresford et al. 1991b). On the contrary, RP67580 displayed about 10–20-fold higher potency in blocking rat NK_1 receptors than guinea pig or human NK_1 receptors (Garret et al. 1991; Barr and Watson 1993; Beaujouan et al. 1993). The existence of pharmacological differences between NK_1 receptors present in rodents, on the one hand, and NK_1 receptors expressed in humans and guinea pigs, on the other, was corroborated with other NK_1 receptor antagonists which were subsequently introduced in this field (see Sect. 3.2), such as GR82334 (Meini et al. 1994) and FK888 (Fujii et al. 1992; Aramori et al. 1994). It should be noted that the ability to recognize with different affinities NK_1 receptors belonging to different species does not extend to all antagonist compounds thus far developed, as demonstrated by the selective antagonist SR140333 (see Sect. 4.3) which displaces ^{125}I-SP specific binding from rat cere-

Table 3 Potencies of tachykinin NK_1 receptor antagonists at tachykinin NK_1, NK_2 and NK_3 receptors measured on isolated smooth muscle preparations

Compound	NK_1 bioassays (pK_B)	NK_2 bioassays (pK_B)	NK_3 bioassays (pK_B)
Spantide	GPI (6.2)[a]	RPA (5.3)[a] HI (4.9)[b]	RPV (<5.0)[a]
Spantide II	GPI (7.1)[a,b] RJV (6.8)[b] RUB (<5.0)[b]	RPA (5.4)[a,b] HT (6.0)[a,b]	RPV (<5.0)[a,b]
L668169	GPI (7.0), (6.4)[b] RJV (6.3)[b] RUB (<5.0)[b] HI (6.3)[b]	RPA (<5.0)[b] HT (6.2)[b] HUB (6.2)[c]	RPV (<5.0)[b]
GR71251	GPI (7.7)[d]	RCMM (<5.0)[e]	RPV (<5.0)[f]
GR82334	GPI (7.6)[f]	RCMM (<5.0)[f]	RPV (<5.0)[f]
FR113680	GPI (7.5)[g], (6.6)[b]	RPA (5.1)[qm] RPA (5.4)[b] RVD (<5.0)[g]	RPV (<5.0)[b,g]
FK888	GPI (9.3)[h] (7.5–8.3)[i] RIS (7.1)[i]	RPA (<5.0)[qm] RVD (<5.0)[i]	RPV (<5.0)[h,qm]
S18523	RVC (9.6)[k]	RPA (5.6)[k]	RPV (5.0)[k]
Cam 2819	GPI (8.4)[l] RJV (8.9)[l]		
GP96345	DCA (8.7)[m] GPI (8.1)[b] RJV (8.3)[b] RUB (6.2)[b]	RPA (<5.0)[b]	RPV (<5.0)[b]
RP67580	GPI (7.2–7.6)[n]	RPA (<6.0)[n]	RPV (<6.0)[n]
SR 140333	GPI (~9.7)[o]	RPA (<6.0)[o]	RPV (<6.0)[o]
GR 203040	GPI (~11.9)[p] DCA (~11.2)[p]		
GR 205171	DCA (11.4)[q]		
LY 303870	RVC (9.4)[r]	RPA (4.7)[r]	RPV (4.7)[r]
CGP 49823	RVC (7.7)[s]	RA (<6.0)[s]	
PD 154075	GPI (9.5)[t]		
TAK 637	GPC (8.3–8.7)[u]	GPC (<7.0)[u]	GPTC (<7.0)[u]

DCA, Dog cerebral arteries; GPI, longitudinal muscle strip of guinea pig ileum; GPTC, guinea pig taenia coli; HI, human ileum; HT, hamster trachea; HUB, hamster urinary bladder; RA, rabbit aorta; RCMM, rat colon muscularis mucosae; RIS, rabbit iris sphincter; RJV, rabbit jugular vein; RPA, rabbit pulmonary artery (endothelium-denuded); RPV, rat portal vein; RUB, rat urinary bladder; RVD, rat vas deferens; RVC, rabbit vena cava.

References: [a] Maggi et al. 1991; [b] Patacchini et al. 1992; [c] Williams et al. 1988; [d] Maggi et al. 1992; [e] Hagan et al. 1990; [f] Hagan et al. 1991; [g] Morimoto et al. 1992; [h] Fujii et al. 1992; [i] Maggi et al. 1994; [j] Wang et al. 1994; [k] Bonnet et al. 1996; [l] McKnight et al. 1994; [m] Snider et al. 1991; [n] Garret et al 1991; [o] Emonds-Alt et al. 1993; [p] Beattie et al. 1995; [q] Gardner et al. 1996; [r] Gitter et al. 1995; [s] Hauser et al. 1994; [t] Boyle et al. 1994; [u] Venkova et al. 2002; [qm] Patacchini et al. unpublished observations.

Table 4 Drawbacks and side effects shown by several tachykinin NK_1 receptor antagonists

Compound	Drawbacks and/or side effects	References
Linear SP analogs bearing dTrp (e.g. spantide)	Low potency; poor selectivity between NK_1 and NK_2 receptors; neurotoxicity; local anesthetic activity; mast cell degranulation activity; blockade of tachykinin unrelated receptors	Folkers et al. 1984; Hoover 1991; Hokfelt et al. 1981; Maggi et al. 1993b; Regoli et al. 1994
GR71251	Mast cell degranulation activity; poor metabolic stability	Hagan et al. 1990, 1991
FR113680	Poor bioavailability and solubility	Hagiwara et al. 1993
Senktide	Inability to penetrate the CNS; interaction with tachykinin unrelated receptors; antagonist activity restricted to rodents	Minami et al. 1988; R. Patacchini, unpublished observations
CP96345	Depression of smooth muscle contractility in various isolated tissues; nonspecific inhibitory effects on neurotransmission; local anesthetic like effects; depression of blood pressure and +/− effects on heart rate	Patacchini et al. 1991; Wang et al. 1994a; Karlsson et al. 1994; Tamura et al. 1993; Lembeck et al. 1992; Constantine et al. 1991
CP99994	Nonspecific reduction of formalin-induced nociceptive responses in gerbils and rats	Smith et al. 1994; Rupniak et al. 1995
RP67580	Nonspecific inhibitory effects on neurotransmission; insufficient penetration of the blood–brain barrier; nonspecific inhibitory effects	Wang et al. 1994a; Holzer-Petsche and Rodorf-Nikolic 1995; Rupniak et al. 1993
FK888 SR140333 CGP49823 LY303870	Poor brain penetration, as shown by their low potency (effective doses 3–10 mg/kg) in preventing foot tapping induced by a SP analog in gerbils, and by their ineffectiveness in preventing cisplatin-induced acute retching in ferrets (at the same doses)	Rupniak et al. 1997
CP122721 GR205171	Brief duration of action at central NK_1 receptors after oral administration	Hale et al. 1998

containing two or three DTrp residues, such as [DPro^4, DTrp^{7,9,10}]SP (Regoli et al. 1984; see Regoli et al. 1994 for review of these analogs).

3.2 NK_1 Receptor-Selective Peptide-Based Antagonists

Historically, the first compound reported to be selective for NK_1 vs. NK_2 and NK_3 receptors was the cyclic antagonist L668169, developed at the end of the 1980s (Williams et al. 1988). L668169, a dodecapeptide bearing a lactam constraint between Gly and Leu and a DTrp residue (Table 2), showed at least tenfold higher potency at guinea pig or rabbit NK_1 receptors as compared to rabbit NK_2 or rat NK_3 receptors, whereas it was apparently inactive at rat NK_1 receptors (Table 3). L668169 was also able to produce weak but selective blockade of contractions produced by SP in the human isolated ileum (Maggi et al. 1992; Table 3).

Soon after the introduction of L668169 a group of researchers at Glaxo (Hagan et al. 1990) presented GR71251 (Table 2), an undecapeptide analog of SP

Table 5 Affinities of tachykinin NK_1 receptor antagonists for NK_1, NK_2 and NK_3 receptors measured in radioligand binding experiments

Compound	NK_1 receptor bioassay (pK_i or pIC_{50})	NK_2 receptor bioassay (pK_i or pIC_{50})	NK_3 receptor bioassay (pK_i or pIC_{50})
FR113680	GPL (pIC_{50}=7.8)[a]	RD (pIC_{50}<5.0)[a]	RCC (pIC_{50}<5.0)[a]
	RCC (pIC_{50}<5.0)[a]		
FK888	GPL (pK_i=9.2)[b]		
	RCC (pK_i=6.3)[b]		
S18523	IM9 (pK_i=9.1)[c]	CHO (pK_i=5.5)[c]	
MLN10930	IM9 (pK_i=9.0)[d]	HUB (pK_i=5.8)[d]	RCC (pK_i<5.0)[d]
	U373MG (pK_i=8.6)[d]		
	RUB (pK_i<5.0)[d]		
MEN11467	IM9 (pK_i=9.4)[e]	CHO (pK_i=6.4)[e]	RCC (pK_i<5.0)[e]
Cam2819	IM9 (pIC_{50}=8.3)[f]		
Sendide	MSC (pK_i=11.4)[g]		
CP96345	BCM (pIC_{50}=8.5)[h]	HUB (pIC_{50}<5.0)[h]	GPC (pIC_{50}<5.0)[h]
	BFM (pIC_{50}=6.6)[h]		
	IM9 (pK_i=9.6)[h]		
CP99994	IM9 (pK_i=9.6)[i]	HUB (pIC_{50}<5.0)[i]	GPC (pIC_{50}<5.0)[i]
CP122721	IM9 (pIC_{50}=9.8)[j]	CHO (pIC_{50}<5.0)[j]	GPC (pIC_{50}<5.0)[j]
CJ11974	IM9 (pK_i=9.7)[k]	CHO (pK_i<6.0)[k]	GPC (pK_i<6.0)[k]
RP67580	RB (pK_i=8.4)[l]	RD (pIC_{50}<5.0)[l]	GPC (pIC_{50}<5.0)[l]
RPR100893	IM9 (pK_i=7.9)[m]		
SR140333	IM9 (pK_i=10.7)[n]	RD (pK_i<6.0)[n]	RCC (pK_i<6.0)[n]
	U373MG (pK_i=9.2)[n]		
	RB (pK_i=10.6)[n]		
SSR240600	IM9 (pK_i=14.2)[o]	CHO (pK_i=7.6)[o]	CHO (pK_i=6.7)[o]
	U373MG (pK_i=10.0)[o]		
	RI (pK_i=9.0)[o]		
GR203040	CHO (pK_i=10.3)[p]	CHO (pK_i<5.0)[p]	GPC (pK_i<6.0)[p]
	U373MG (pK_i=10.5)[p]		
	RCC (pK_i=8.6)[p]		
GR205171	CHO (pK_i=10.6)[q]	RC (pK_i<5.0)[q]	GPC (pK_i<5.0)[q]
	RCC (pK_i=9.5)[q]		
	FCC (pK_i=9.8)[q]		
L733060	CHO (pIC_{50}=9.0)[r]		
L742694	CHO (pIC_{50}=10.0)[s]	CHO (pIC_{50}=5.2)[s]	CHO (pIC_{50}=6.8)[s]
L754030	CHO (pIC_{50}=10.0)[t]	CHO (pIC_{50}<6.0)[t]	CHO (pIC_{50}<7.0)[t]
L732138	CHO (pIC_{50}=8.6)[u]	CHO (pIC_{50}<6.0)[u]	CHO (pIC_{50}<6.0)[u]
L737488	CHO (pIC_{50}=9.8)[v]	CHO (pIC_{50}<6.0)[v]	CHO (pIC_{50}<6.0)[v]
LY303870	IM9 (pK_i=9.8)[w]	CHO (pK_i<6.0)[w]	GPC (pK_i<6.0)[w]
	^{3}H-Gaud (pK_i=10.0)[w]		
CGP49823	BR (pIC_{50}=7.9)[x]	BB (pIC_{50}=5.0)[x]	GC (pIC_{50}=5.6)[x]
SDZNKT343	COS-7 (pK_i=9.2)[y]	COS-7 (pK_i=6.3)[y]	COS-7 (pK_i=5.5)[y]
NKP608	BR (pIC_{50}=8.6)[z]	BB (pIC_{50}=5.7)[z]	GC (pIC_{50}=5.8)[z]
RP154075	IM9 (pK_i=9.5)[aa]	HUB (<5.0)[bb]	CHO (<6.0)[bb]
TAK637	IM9 (pIC_{50}=9.3)[cc]		GPC (pIC_{50}<6.0)[cc]
R116301	CHO (pK_i=9.3)[dd]	CHO (pK_i=6.1)[dd]	CHO (pK_i=7.0)[dd]

BB, Bovine bladder membranes; BCM, bovine caudate membranes; BR, bovine retina membranes; CHO, Chinese hamster ovary cells bearing transfected human NK_1, NK_2 or NK_3 receptors; COS-7, cell line bearing transfected human NK_1, NK_2 or NK_3 receptors; FCC, ferret cerebral cortex; GC, gerbil cortical mem-

incorporating a bicyclic conformational constraint designed to eliminate NK_1 receptor-activating conformations. GR71251 was endowed with good potency at NK_1 receptors and selectivity vs. NK_2 and NK_3 receptors (Table 3), but it was shown to release histamine from rat peritoneal mast cells (Hagan et al. 1990). This side effect, along with rapid metabolic degradation (Hagan et al. 1991), limited the use of GR71251 (Table 4). These problems were overcome with the synthesis of GR82334, an undecapeptide compound bearing the same substitutions introduced in GR71251, but in this case on the backbone of the nonmammalian tachykinin physalaemin (Hagan et al. 1991; Table 2). GR82334 retained the same potency and selectivity for NK_1 receptors as GR71251 (Table 3) and, being devoid of important side effects and being resistant to metabolic degradation, became a very useful tool for both in vitro (e.g., Guo et al. 1995) and in vivo (e.g., Hagan et al. 1991; Beresford et al. 1991a) studies (see also Table 1).

A systematic approach was undertaken by researchers at Fujisawa in the early 1990s to identify the minimal sequence contained in the octapeptide SP antagonist [DPro^4 DTrp^{7,9,10} Phe^{11}]SP(4–11) that was capable of binding with sufficient affinity to tachykinin receptors. After a subsequent lead optimization, low-molecular weight peptide fragments endowed with high affinity and selectivity for NK_1 receptors were identified, the most interesting being the tripeptide FR113680 (Morimoto et al. 1992; Table 2). FR113680 displayed a fairly good affinity for guinea pig NK_1 receptors, whereas it was inactive/much less active at rat NK_1 receptors and at NK_2 and NK_3 receptors (Tables 3, 5). In in vivo experiments, FR113680 (1–10 mg/kg intravenously) fully prevented both bronchoconstriction and airway edema induced by SP, and only partially the effects produced by NKA or capsaicin, thought to be mediated by both NK_2 and NK_1 receptors (Murai et al. 1992). Nevertheless, FR113680 was not developed as antiasthmatic drug by Fujisawa, because of its unfavorable chemico-physical characteristics (Hagiwara et al. 1993; Table 4). Rather, a new series of short peptides was synthesized that had reduced weight and lipophilicity: the most promising compound of this series was FK888 (Table 2; Fujii et al. 1992). FK888 showed

branes; GPC, guinea pig cortex membranes; GPL, guinea pig lung membranes; Hcaud, human caudate homogenate; HUB, hamster urinary bladder cell membranes; IM9, human lymphoblastoma cell line membranes; MSC, mouse spinal cord membranes; NE, not effective; RB, rat brain membranes; RCC, rat cerebral cortex membranes; RD, rat duodenum smooth muscle membranes; RFM, rat forebrain membranes; RI, rat ileum; RUB, rat urinary bladder cell membranes; U373MG, human astrocytoma cell line membranes.

References: [a] Morimoto et al. 1992; [b] Fujii et al. 1992; [c] Bonnet et al. 1996; [d] Astolfi et al. 1997; [e] Cirillo et al. 2001; [f] McKnight et al. 1994; [g] Sakurada et al. 1992; [h] Snider et al. 1991; [i] McLean et al. 1993); [j] McLean et al. 1996; [k] Tsuchiya et al. 2002; [l] Garret et al. 1991; [m] Fardin et al. 1994; [n] Emonds-Alt et al. 1993; [o] Emonds-Alt et al. 2002; [p] Beattie et al. 1995; [q] Gardner et al. 1996; [r] Harrison et al. 1994; [s] Hale et al. 1996; [t] Hale et al. 1998; [u] Cascieri et al. 1994; [v] MacLeod et al. 1995; [w] Gitter et al. 1995; [x] Hauser et al. 1994; [y] Walpole et al. 1998a; [z] Vassout et al. 2000; [aa] Boyle et al. 1994; [bb] Singh et al. 1997; [cc] Natsugari et al. 1999; [dd] Megens et al. 2002.

high affinity for (guinea pig) NK_1 receptors and remarkable selectivity vs. rat NK_1, NK_2 and NK_3 receptors (Tables 3, 5). Furthermore, FK888 was found to be active in blocking airway edema, plasma protein extravasation and other responses produced by exogenous and endogenous tachykinins in in vivo bioassays, even after oral administration (Table 6; Fujii et al. 1992; Murai et al. 1993; Hirayama et al. 1993; Wang et al. 1994b). Owing to its potency and lack of nonspecific effects, FK888 was regarded as promising candidate for the treatment of asthma in clinical trials. However, FK888 failed to afford beneficial effects on exercise-induced bronchoconstriction in asthmatics, even though it significantly shortened the recovery times, suggesting a possible role of tachykinin NK_1 receptors in the late bronchoconstrictor response to exercise (Ichinose et al. 1996).

A strict analog of FK888 is represented by the Servier compound S18523: a dipeptide bearing a tetrazolyl-butyl group on the indole nitrogen of FK888 (Table 2; Bonnet et al. 1996). S18523 shows highly improved water solubility compared to FK888 and slightly higher affinity for human and rabbit NK_1 receptors (Tables 3, 5; Bonnet et al. 1996). In vivo, S18523 exerts potent antinociceptive effects in classical pain tests (hot-plate in mice, phenylbenzoquinone-induced writhing in mice and formalin test in rats), and blocks SP- or capsaicin-induced plasma protein extravasation in guinea pigs, also after oral administration.

Another example of a short peptide endowed with high potency and selectivity for the NK_1 receptor is represented by MEN10930, a tripeptide analog of FK888 synthesized at Menarini Laboratories (Table 2; Astolfi et al. 1997). MEN10930 potently and selectively bound to human transfected NK_1 receptors, whereas it showed approximately 10,000-fold lower affinity for the rat NK_1 receptor subtype (Table 5). Further modifications of MEN10930 led to discovery of a partially retro-inverse peptidomimetic antagonist: MEN11467 (Table 2). MEN11467 is characterized by the presence of a diaminocyclohexane moiety and of a (2-naphthyl)alanine residue, inserted in a reversed direction starting from the C terminus. These modifications preserve MEN11467 from enzymatic degradation, while the high affinity for the NK_1 receptor is conserved (Cirillo et al. 2001). MEN11467 showed high affinity and selectivity for human NK_1 receptors in binding experiments, with clearly insurmountable kinetics of action (Cirillo et al. 2001; Table 5). In vivo, oral administration of MEN11467 produced a long-lasting inhibition of both bronchoconstriction and plasma protein extravasation induced in anesthetized guinea pigs by a selective NK_1 receptor agonist (Cirillo et al. 2001; Table 6). MEN11467 was also able to prevent albumin-induced mucus secretion in sensitized ferrets (Khan et al. 2001) and was therefore proposed for development as an antisecretory drug in allergic asthma. In contrast to its peripheral activity, MEN11467 showed poor ability to penetrate into the central nervous system (CNS) and block central NK_1 receptors (Cirillo et al. 2001).

An approach similar to that of Fujisawa–that is identification of a short peptide sequence bearing appreciable affinity for tachykinin receptors followed by optimization–was followed by a group of researchers at Parke-Davis, leading to

Table 6 Preclinical in vivo effects of tachykinin NK_1 receptor antagonists being of possible therapeutic relevance

Compound	Effect	Dosage	Therapeutic perspective
FK888	Blockade of SP-induced airway edema[a]	0.011 mg/kg i.v. 4.2 mg/kg p.o.	Antiasthmatic
	Blockade of SP-induced plasma exudation in guinea pig lower trachea and bronchi[b]	0.1 μmol/kg i.v.	Antiasthmatic
MEN1467	Blockade of [Sar^9] SPsulfone-induced bronchoconstriction in anesthetized guinea pig	29 μg/kg i.v.	Antiasthmatic
	Blockade of [Sar^9] SPsulfone-induced plasma protein extravasation in guinea pig bronchi	6.7 mg/kg p.o.	Antiasthmatic
CP96345	Mild thermal analgesia in mice (hot plate test)[d]	30 mg/kg i.p.	Analgesic
	Blockade of SP- or noxious heat-induced tail flick in rats	5 mg/kg s.c.	Analgesic
	Blockade of plasma protein extravasation in the rat hind paw induced by various irritants	3–9 μmol/kg i.v.	Antiinflammatory
	Blockade of plasma protein extravasation induced by vagus nerve stimulation or capsaicin in guinea pigs[e] (see text for further examples)	100 nmol/kg i.v.	Antiinflammatory
CP99994	Blockade of plasma protein extravasation induced by capsaicin in guinea pigs[f]	3–30 mg/kg p.o.	Antiinflammatory
	Prevention of tracheal vascular permeability induced by hypertonic saline or capsaicin in rats	1–4 mg/kg i.v.	Antiinflammatory
	Prevention of foot tapping in gerbils elicited by i.t. GR73632[g]	0.1–1 mg/kg s.c.	Analgesic
	Attenuation of cisplatin-induced emesis in the ferret[h]	0.3–3 mg/kg i.v.	Antiemetic
	Reduction of vomiting response to $CuSO_4$ and apomorphine in the dog (see text for further examples)[i]	40 μg/kg bolus plus 0.3 mg/kg/h i.v.	Antiemetic
RP67580	Reduction of writhings in phenylbenzo-quinone-injected mice[m]	70 μg/kg s.c.	Analgesic
	Reduction of paw lickings in formalin-injected mice[m] (see text for further examples)	3.7 mg/kg s.c.	Analgesic
RPR100893	Blockade of plasma protein extravasation within the dura mater elicited by electrical stimulation of the trigeminal ganglion in guinea pigs[n]	0.01–100 μg/kg p.o.	Antimigraine
SR140333	Reduction of fecal mass excretion and diarrhea in castor oil-pretreated rats[o]	0.02–20 μg/kg s.c.	Antidiarrheal
	Blockade of secretory responses evoked by alphaIgE or capsaicin in the human colonic mucosa (note: in vitro study)[p]		Antidiarrheal in food allergy and inflammatory bowel disease
GR203040	Antagonism of SPOMe-induced reduction of carotid vascular resistance in rabbits[q]	1–100 μg/kg i.v.	Antimigraine
	Prolonged antiemetic activity against radiation, cisplatin and other stimuli in the ferret[r]	0.03–0.3 mg/kg (s.c. or p.o.)	Antiemetic
GR205171	Antiemetic activity against X-radiation in the ferret[s]	0.1 mg/kg p.o.	Antiemetic
	Antiemetic activity against ipecacuanha in dogs[t]	0.2 mg/kg p.o.	Antiemetic
L733060	Abolition of vocalizations elicited by i.c.v. infusion of GR73632 in guinea pigs[u]	3 mg/kg s.c.	Antidepressant
	Abolition of maternal separation-induced vocalizations in guinea pig pups[v]	3 mg/kg s.c.	Antidepressant

Table 6 (continued)

Compound	Effect	Dosage	Therapeutic perspective
LY303870	Reduction of the severity of inflammatory bowel disease (IBD) in mice and facilitation of partial healing of lesions in mice with pre-existing IBD[w]		Antiinflammatory in inflammatory bowel disease
CGP49823	Increase in active social time and reduction of the immobility time of rats in the swim test[x]	3–30 mg/kg p.o.	Anxiolytic antidepressant
NKP608	Increase in the time spent by rats in social contact in a social interaction test	0.01–1 mg/kg p.o.	Anxiolytic
	Increase in the time spent by the intruder rat in social contact with the resident rat in a social exploration test[y]	0.03–3 mg/kg p.o.	Anxiolytic
PD154075	Prevention of both thermal and mechanical hypersensitivity induced by surgery in rats[z]	3–100 mg/kg s.c.	Antihyperalgesic in postoperative pain
	Blockade of hypersensitivity induced by chronic constriction injury (sciatic nerve ligation) in both rats[aa] and guinea pigs[bb]	10–100 mg/kg s.c. 0.1 mg/kg p.o.	Analgesic in neuropathic pain
TAK637	Increase in the volume threshold required to elicit micturition reflex in awake guinea pigs[cc]	0.01 mg/kg p.o.	Control of urinary incontinence
	Decrease in the number of rhythmic bladder contractions elicited by infusion of saline	1 mg/kg i.v.	
	Reduction of capsaicin-induced micturition reflex in guinea pigs	0.03–0.3 mg/kg i.v.	
	Reduction of restraint-stress-stimulated fecal pellet output in gerbils[dd]	0.1–1 mg/kg p.o.	Control of irritable bowel syndrome
	Reduction of the number of distension-induced abdominal contractions in rabbits previously subjected to colonic irritation[ee]	0.1–3 mg/kg i.d.	

i.c.v., Intracerebroventricular; i.d., intraduodenal; i.p., intraperitoneal; i.t., intrathecal; i.v., intravenous; s.c., subcutaneous; p.o., per os.

References: [a] Fujii et al. 1992; [b] Hirayama et al. 1993; [c] Cirillo et al. 2001; [d] Lecci et al. 1991; [e] Yashpal et al. 1993; [f] Lembeck et al. 1992; [g] Lei et al. 1992; [h] McLean et al. 1993; [i] Piedimonte et al. 1993; [j] Rupniak et al. 1995; [k] Tattersall et al. 1993; [l] Watson et al. 1995; [m] Garret et al. 1991; [n] Lee et al. 1994; [o] Croci et al. 1997; [p] Moriarty et al. 2001; [q] Beattie et al. 1995; [r] Gardner et al. 1995; [s] Gardner et al. 1996; [t] Kramer et al. 1998; [u] Sonea et al. 2002; [v] Vassout et al. 1994; [w] Vassout et al. 2000; [x] Gonzalez et al. 1998; [y] Gonzalez et al. 2000; [z] Doi et al. 1999; [aa] Doi et al. 2000; [bb] Okano et al. 2001; [cc] Okano et al. 2002.

development of a number of peptoid compounds, the most potent of which was Cam2819 (Table 2). Cam2819 showed nanomolar affinity for guinea pig, rabbit and human NK_1 receptors (Tables 3, 5; McKnight et al. 1994).

In 1992 Sakurada and coworkers reported the identification (from random screening) of an extremely potent antagonist of SP: sendide (or selective NK_1 receptor D-amino acid-containing peptide; Table 2). Sendide showed a subnanomolar affinity for NK_1 receptors in the mouse spinal cord (Table 6). Selectivity of sendide for NK_1 over NK_2 and NK_3 receptors was claimed on the basis of an in vivo experiment in which sendide (intrathecally administered) was at

least 100-fold more potent in preventing SP from inducing scratching, biting and licking in the mouse than in preventing NKA or NKB from eliciting these responses (Sakurada et al. 1992). Subsequently, sendide was shown to produce antinociceptive effects in the mouse paw formalin test (Sakurada et al. 1995) and antiemetic effects in the cisplatin model of emesis in the ferret (Minami et al. 1998). However, the utility of sendide as NK_1 receptor antagonist was hampered by several factors, including its poor activity at NK_1 receptors present in species different from the mouse (Table 4).

4
Nonpeptide Antagonists

4.1
CP96345 and Related Compounds

The era of nonpeptide antagonists for the NK_1 receptor of tachykinins began in 1991, when the first one of this series, CP96345, was reported by Pfizer (Snider et al. 1991). The introduction of CP96345 gave rise to plenty of structure–activity studies all over the world, leading to discovery of new nonpeptide compounds, and to plenty of studies aiming at investigating the pathophysiological role of the NK_1 receptor by the use of this new potent antagonist. CP96345 (Fig. 1) is a quinuclidine derivative, discovered by the screening of a chemical collection followed by lead optimization (Lowe et al. 1992). CP96345, in its active form [the (2*S*,3*S*)-enantiomer], displays high affinity for NK_1 receptors present in human, guinea pig, rabbit and other human-related species, and remarkable selectivity over NK_2 and NK_3 receptors. In contrast, it recognizes with much less affinity NK_1 receptors of rodents (Tables 3 and 5). These observations were instrumental for recognition of the existence of species-related heterogeneity of the NK_1 receptor (see Sect. 2.1.1). From the many in vivo studies in which CP96345 was used, the role played by NK_1 receptors in nociception received strong support (Table 6). For example, (±)-CP96345 selectively blocked the aversive behavior induced by intrathecal SP in mice, and also produced mild analgesic effects in the hot plate test (Lecci et al. 1991). CP96345 prevented excitation of dorsal horn neurons in cat spinal cord following application of noxious heat to the appropriate receptive field in the hind limb (Radhakrishan and Henry 1991). In other studies, CP96345 was proven to inhibit carrageenin-induced mechanical hyperalgesia in rats (Birch et al. 1992), paw licking induced by formalin in mice (Sakurada et al. 1993), abdominal stretching induced by intracolonic instillation of acetic acid in mice (Nagahisa et al. 1992), tail flick responses induced by noxious thermal and chemical stimuli in the rat (Yashpal et al. 1993) and vocalization induced by mechanical stimuli of mononeuropathic and diabetic rats (Coudoré-Civiale et al. 1998).

Unequivocal evidence for the involvement of NK_1 receptors in mediating inflammatory responses was provided by means of CP96345 (Table 6). For example, CP96345 was found effective in preventing plasma protein extravasation

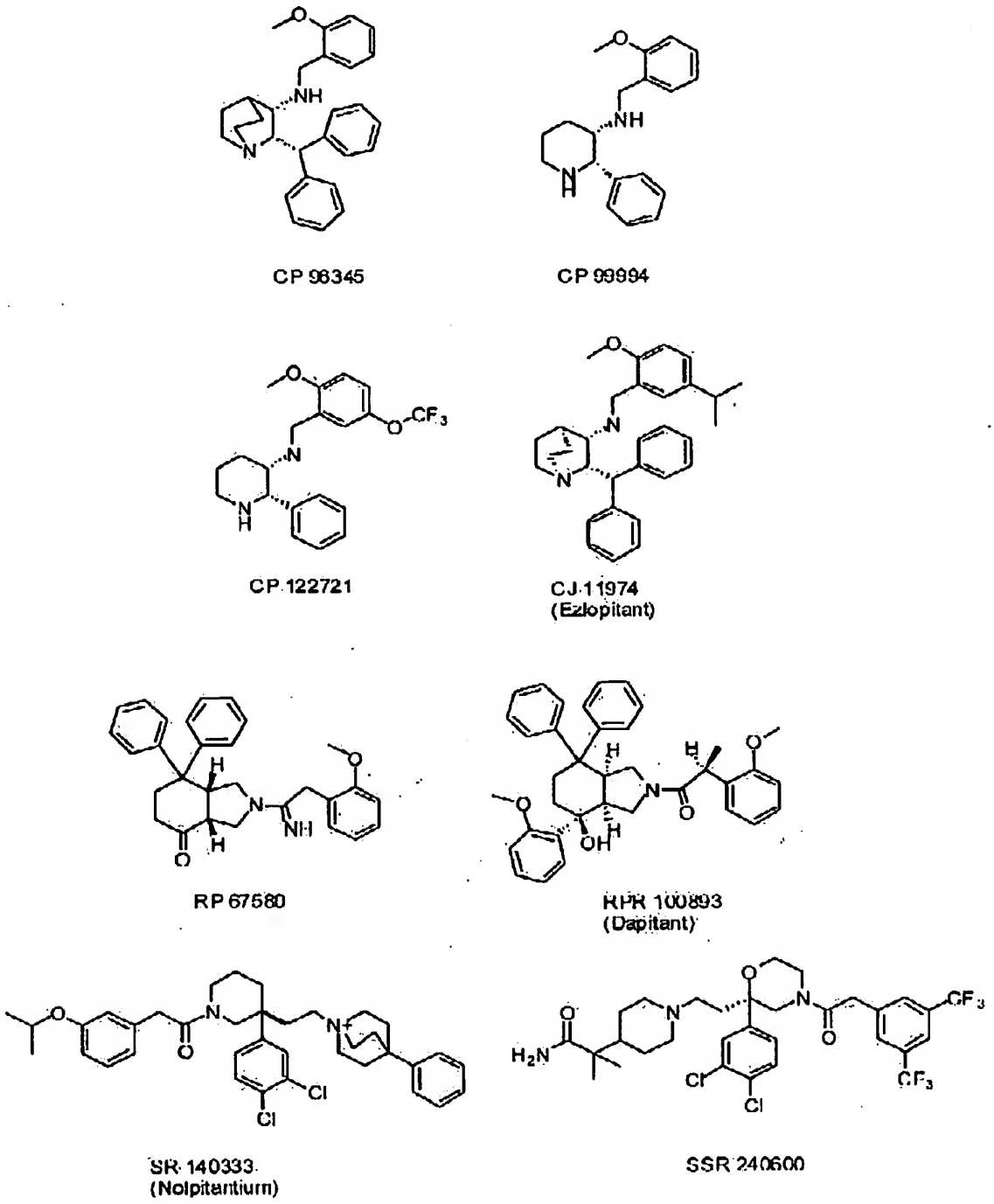

Fig. 1 Chemical structures of nonpeptide tachykinin NK_1 receptor antagonists: I

and/or edema caused by several pro-inflammatory stimuli such as capsaicin in the rat urinary bladder (Eglezos et al. 1991), SP, mustard oil and stimulation of the saphenous nerve in the rat hind paw skin (Lembeck et al. 1992) as well as cigarette smoke in the rat trachea (Delay-Goyet and Lundberg 1991; Delay-Goyet et al. 1992). Likewise CP96345 prevented neurogenic plasma exudation evoked

by electrical stimulation of the cervical vagus and by intravenous (i.v.) capsaicin in guinea pigs (Lei et al. 1992). CP96345 (administered chronically) has recently been shown to attenuate significantly colonic inflammation and oxidative stress produced by dextran sulfate in rats: an animal disease model resembling chronic ulcerative colitis in humans (Stucchi et al. 2000). Although not described in the original reports on CP96345 pharmacology, several nonspecific effects produced by this compound, both in vitro and in vivo, were reported (Table 4). Subsequent investigations of these nonspecific effects showed that they are due to interaction of CP96345 with several ion channels, including L- and N-type calcium channels (Schmidt et al. 1992; Guard et al. 1993), and voltage-dependent sodium channels (Caesar et al. 1993). Two relevant observations dealing with nonspecific activities of CP96345 are worth mentioning: (a) these effects are equally exerted by either the (+) (named: CP96344) or (−) isomer of CP96345, while the NK_1 receptor affinity is present in the (−) isomer only, and (b) they are evident at micromolar concentrations. It follows that, due to the reduced affinity of CP96345 for rat and mouse NK_1 receptors (compared to that for NK_1 receptors of other species), the window of selectivity between doses of CP96345 producing specific (i.e., NK_1 receptor-mediated) vs. nonspecific (i.e., membrane channel-mediated) effects is narrow in the rodent species. Thus, the introduction of the (+) isomer (CP96344) as control drug in studies in which CP96345 is used, is necessary to avoid misinterpretation of the results.

In an attempt to overcome the many drawbacks of CP96345, Pfizer researchers synthesized CP99994, a compound in which the quinuclidine ring of CP96345 was replaced by a piperidine, and the benzhydryl moiety by a benzyl group (Fig. 1; McLean et al. 1993). Only the (2*S*, 3*S*) isomer of CP99994 (like CP96345) shows considerable affinity for NK_1 receptors, whereas the (2R, 3R) enantiomer (named CP100263) is more than 1000-fold weaker at NK_1 receptors. CP99994 shows high affinity for human NK_1 receptors comparable to that of CP96345 (Table 5), but a 100-fold reduced affinity (IC_{50}=3 μM) for L-type calcium channels relative to CP96345 (IC_{50}=27 nM) (McLean et al. 1993). Similarly to CP96345, CP99994 showed marked antinociceptive activity and was a potent inhibitor of plasma protein extravasation in the airways (Table 6). However, some nonspecific effects of CP99994 (i.e., effects shared by its inactive enantiomer CP100263) in antinociceptive tests in the rat, mouse and gerbil have been reported (Table 4; Smith et al. 1994; Rupniak et al. 1995). In this regard it is worth to mention the study of Lombet and Spedding (1994) who showed CP99994 to interact with phenylalkylamine calcium channels in rat skeletal muscle membranes, with quite the same potency of CP96345 (K_i=0.17 vs. 0.94 μM, respectively), indicating that even CP99994 may have the potential of producing 'nonspecific' effects under certain circumstances. On the basis of its preclinical profile, CP99994 has been used in clinical trials to prove its analgesic and antiasthmatic properties, obtaining conflicting results. Dionne et al. (1998) reported that CP99994 reduced postoperative pain in patients undergoing dental extraction, although the nonsteroidal antinflammatory agent, ibuprofen, was more effective. On the other hand, CP99994 failed to produce significant analgesic activ-

ity in patients suffering from peripheral neuropathy (Suarez et al. 1994), and was proven unable to prevent bronchoconstriction and cough induced by hypertonic saline in mild asthmatic patients (Fahy et al. 1995).

CP99994 was the first NK_1 receptor antagonist to be used for demonstrating that blockade of NK_1 receptors produces antiemetic effects against a broad variety of emetogenic stimuli (Table 6). In 1993 Bountra et al. and Tattersall et al. (1993) reported that CP99994 is effective in the ferret in attenuating emesis induced by cisplatin, morphine, ipecacuana, copper sulfate, radiation and other cytotoxic drugs. Subsequently the antiemetic activity of CP99994 was proven in the dog (Watson et al. 1995), *Suncus murinus* (Tattersall et al. 1995) and cat (Lucot et al. 1997) against a variety of central, peripheral and mixed central and peripheral emetic stimuli. An interesting follow-up of CP99994 was CP122721 (Fig. 1), an analog bearing an additional trifluoromethoxy group in the benzyl ring of the molecule. CP122721 shows similar high affinity for human NK_1 receptors as CP99994 (Table 5) and 1000-fold reduced ability to interact with calcium channels than its parent compounds, CP96345 and CP99994 (McLean et al. 1996). The analysis of the kinetics of interaction of CP122721 with the NK_1 receptor indicated a noncompetitive/insurmountable behavior of this antagonist (McLean et al. 1996). CP122721 was consistently more potent than CP99994 in blocking capsaicin-induced plasma protein extravasation in guinea pig ureter and lung: the greater potency of CP122721 was attributed to its improved bioavailability and to its insurmountable mechanism of action (McLean et al. 1996). Like CP99994, CP122721 was shown to be a potent inhibitor of both retching and vomiting elicited by a broad spectrum of emetic agents in ferrets (Gonsalves et al. 1996). The efficacy of CP122721 has been confirmed in clinical trials in which this compound significantly attenuated delayed emesis due to cisplatin chemotherapy (Kris et al. 1997) and postoperative nausea and vomiting (Gesztesi et al. 1998, 2000).

A further development of Pfizer compounds is represented by CJ11974, or ezlopitant, a quinuclidine analog of CP96354 bearing an additional isopropyl group in the benzyl ring (Fig. 1). CJ11974 is endowed with high affinity and selectivity for human NK_1 receptors, and oral efficacy in preventing cisplatin-induced emesis in ferrets (Tsuchiya et al. 2002; Table 5). The therapeutic potential of this compound has been documented in Phase II clinical trials (Evangelista 2001). CJ11974 proved to be effective in controlling chemotherapy-induced emesis (Hesketh et al. 1999), but as it was less effective for nausea it was discontinued for this indication (Evangelista 2001). Subsequently, CJ11974 has been shown to reduce emotional responses to rectosigmoid distension and related symptoms in patients affected by irritable bowel syndrome (IBS) (Lee et al. 2000), and consequently it is currently being developed for this indication (Evangelista 2001).

4.2
RP67580 and Related Compounds

Soon after the introduction of the first nonpeptide NK_1 receptor antagonist, CP96345, another interesting compound was described by researchers at Rhone-Poulenc: RP67580 (Garret et al. 1991). As discussed before (see Sect. 2.1.1) RP67580 has been instrumental in unraveling the species-dependent variations in the pharmacology of NK_1 receptor antagonists, being significantly more potent at rat or mouse than guinea pig or human NK_1 receptors. RP67580 is a perhydroisoindolone derivative (Fig. 1), whose affinity for NK_1 receptors (Table 5) is confined to the (3aR, 7aR) enantiomer, whereas the (3a*S*, 7a*S*) enantiomer (or RP 68651) is inactive up to 10 μM (Garret et al. 1991). RP67580 showed good selectivity relative to NK_2 and NK_3 tachykinin receptors (Tables 3 and 5) and was found to be as potent as morphine in two classical tests for analgesia: phenylbenzoquinone-induced writhing and formalin test (Garret et al. 1991; Table 6). Further in vivo studies confirmed the ability of RP67580 to produce stereoselective analgesic/antiinflammatory effects against various stimuli. As an example, RP67580 attenuated chronic hyperalgesia in streptozotocin-induced diabetic rats (Courtiex et al. 1993), prevented capsaicin-induced plasma protein extravasation in the dura mater of guinea pigs (Moussaoui et al. 1993) and plasma protein extravasation in the dura mater of rats elicited by electrical stimulation of the trigeminal ganglion (Shepheard et al. 1993). Holzer-Petsche and Rordorf-Nikolic (1995) noticed that intrathecal RP67580 was able to inhibit reflex changes of mean arterial pressure and intragastric pressure in response to systemic capsaicin administration in anesthetized rats, whereas intravenous administration of RP67580 was not, concluding that this could be due to insufficient penetration of this antagonist through the blood–brain barrier (Table 4). A further drawback of RP67580 is its ability to interact with various calcium channels at submicromolar to micromolar concentrations (Lombet and Spedding 1994; Rupniak et al. 1993). Although it remains to be clarified whether this calcium blocking activity of RP67580 resides equally in the two enantiomers, it has been argued that this mechanism could be responsible for acute antinociceptive effects produced by RP67580 in several animal models of pain. The former conclusion is supported by the observation that the channel blockers nifedipine and verapamil are as effective as RP67580 in one of these models (formalin paw test in gerbils; Rupniak et al. 1993).

The recognition of the existence of species-dependent variations of the NK_1 receptor, and the observation that RP67580 was more potent on the rodent type rather than on the human type, prompted Rhone-Poulenc researchers to undertake structure–activity studies in order to improve the selectivity of their compound toward the human type NK_1 receptor. The most interesting outcome of this search was the nonpeptide RPR100893 (Fardin et al. 1994), a product bearing an additional aromatic ring in the RP67580 structure: a modification leading to increasing structural complexity of the molecule (i.e., two additional stereogenic centers) (Fig. 1). RPR100893 has 100-fold higher affinity for human NK_1

receptors than for rat or mouse receptors (Fardin et al. 1994) (Table 5), showing time-dependent kinetics in its binding to the human receptor, and insurmountable antagonism of SP-induced inositol phosphate production in cultured human U373MG astrocytoma cells (Fardin et al. 1994). In vivo, RPR 100893 showed good oral bioavailability and efficacy in preventing plasma protein extravasation induced by either tachykinins or capsaicin in guinea pig trachea, and in reducing formalin-induced nociceptive behavior in guinea pigs (Moussaoui et al. 1994). In particular, Lee et al. (1994) demonstrated the ability of RPR100893 to block plasma protein extravasation within the dura mater and conjunctiva of guinea pigs induced by capsaicin or electrical stimulation of the trigeminal ganglion, and suggested that this compound (or others of the same series) could prove to be useful for treating migraine and cluster headaches (Table 6). This latter hypothesis was further supported by Cutrer et al. (1995) who showed RPR100893 capable of selectively reducing c-*fos* expression in the trigeminal nucleus caudalis in guinea pigs, elicited by intracisternal injection of capsaicin. However, subsequent clinical trials with RPR100893 have failed to show any efficacy against migraine pain (Rupniak and Kramer 1999).

4.3
SR140333 and Related Compounds

In 1993 Emonds-Alt and coworkers at Sanofi discovered the piperidine-based compound SR140333 (Fig. 1), one of the most potent and selective NK_1 receptor antagonists since then developed (Table 5). SR140333 was reported to possess subnanomolar affinity for NK_1 receptors of various species, including human, guinea pig and rat: thereby showing no preference for any of the two NK_1 receptor 'families' previously identified by the use of other antagonists (see Sect. 2.1.1; Emonds-Alt et al. 1993). As for other nonpeptide antagonists containing stereogenic centers in the chemical structure, the affinity of SR140333 for NK_1 receptors was (much) higher in one enantiomer, [the (*S*) enantiomer], than in the corresponding (*R*) enantiomer (or SR140603). SR140333 was proven to be a highly potent antagonist at NK_1 receptors present in classical isolated smooth muscle preparations such as the guinea pig ileum and the rabbit pulmonary artery. However, in these bioassays SR140333 produced insurmountable effects, probably due to the slow rate of reversibility of its receptor interaction (Emonds-Alt et al. 1993; Table 3). In vivo, SR140333 potently [50% effective dose (ED_{50}) ranging from 3 to 42 μg/kg i.v.] antagonized hypotension, bronchoconstriction and plasma protein extravasation in the skin elicited by selective NK_1 receptor agonists in dogs, guinea pigs and rats, respectively (Emonds-Alt et al. 1993). Subsequent investigations aiming at clarifying the role of NK_1 receptors in nociception and neurogenic inflammation demonstrated the efficacy of SR140333 in various animal models of pain and inflammation. As an example, SR140333 reduced the mouse ear edema provoked by topical capsaicin application (Inoue et al. 1996), abolished the tail-flick reflex facilitation induced by noxious heat in rats (Jung et al. 1994), abolished the cutaneous edema induced

by electrical stimulation of the rat saphenous nerve (Towler and Brain 1998), reduced colonic inflammatory responses induced by trinitrobenzene sulfonic acid in the rat (Mazelin et al. 1998) and produced a long-lasting inhibition of mustard oil-induced plasma protein extravasation in the dorsal skin of the rat hind paw (Amann et al. 1995). However, Amann and coworkers (1995) also reported that SR140333, at doses that caused inhibition of neurogenic inflammation, was unable to prevent the acute behavioral responses elicited in conscious rats by chemical irritants (capsaicin, PGE_2) or noxious heat. In an in vivo model of chemically induced diarrhea, Croci et al. (1997) have shown that systemic pretreatment with SR140333 may (partially) reduce both fecal mass excretion and the rate of diarrhea in castor-oil treated rats, without producing constipation in normal, untreated rats. More recently, the potential use of SR140333 as an antidiarrheal drug for food allergy or IBS has been supported by the ability of SR140333 to reduce the secretory responses evoked in the human colonic mucosa by either mast cells or by primary afferent neuron stimulation (Moriarty et al. 2001; Table 6).

A follow-up compound of SR140333 is SSR240600 (Emonds-Alt et al. 2002; Fig. 1). SSR240600 maintains a very high affinity for the human NK_1 receptor present in different human cell lines (Table 5), comparable to that shown by the parent compound SR140333. However SSR240600, unlike SR140333, can distinguish among species, being consistently less potent on rat and gerbil NK_1 receptors than on human or guinea pig receptors (Emonds-Alt et al. 2002). Like SR140333, SSR240600 has been reported to produce insurmountable antagonism of NK_1-receptor mediated responses obtained in isolated preparations, and long-lasting inhibitory effects against hypotension, bronchoconstriction and plasma protein extravasation elicited by selective NK-1 receptor agonists in dogs and guinea pigs, respectively (Emonds-Alt et al. 2002). Moreover, SSR240600 was able to prevent citric acid-induced cough in guinea pigs (whereas SR140333 was not): an effect claimed to be due to its easy penetration into the brain (Emonds-Alt et al. 2002). Concomitantly, Steinberg et al. (2002) have reported that SSR240600 inhibits distress vocalization induced by maternal separation in guinea pig pups and counteracts the increase in body temperature induced by isolation stress: two effects that suggest a potential antidepressant-like activity of this compound, as shown previously for certain Merck compounds found active in similar animal models (see Sect. 4.5).

4.4 GR203040 and Related Compounds

Structure–activity studies on the backbone of the Pfizer compound CP99994 were undertaken by researchers at Glaxo, aiming at improving oral bioavailability, metabolic stability and specificity of action of this molecule. Their efforts lead to the discovery of a tetrazole-substituted analog of CP99994, named GR203040 (Fig. 2; Ward et al. 1995; Beattie et al. 1995). GR203040 showed slightly higher affinity for human NK_1 receptors expressed by different cell lines than

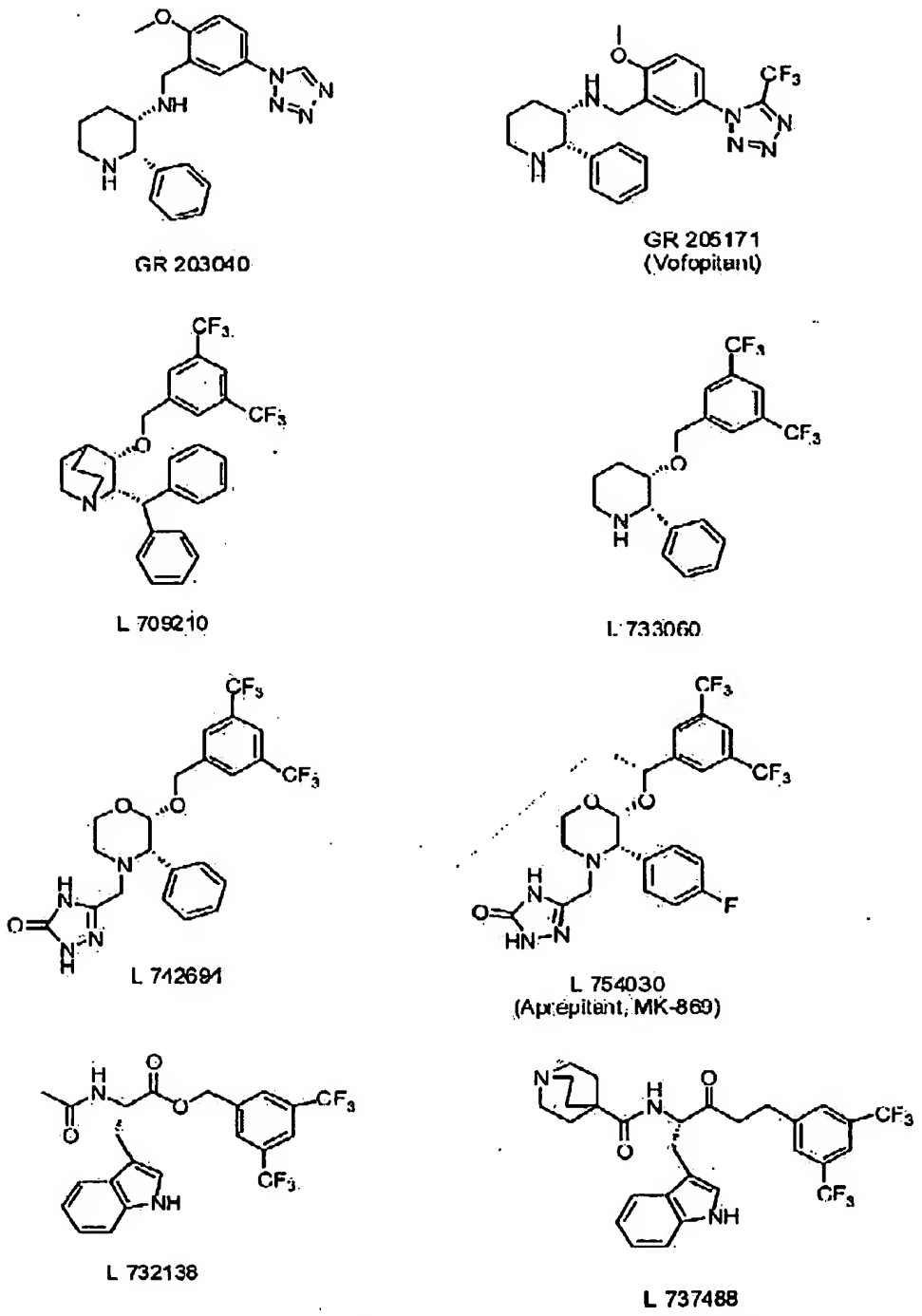

Fig. 2 Chemical structures of nonpeptide tachykinin NK_1 receptor antagonists: II

the parent compounds CP99994 and CP96345, whereas at rat NK_1 receptors GR203040 was approximately 50-fold less potent than at human receptors, as observed for CP99994 and CP96345 (see Table 5; Beattie et al. 1995). As for the Pfizer compounds, the affinity for NK_1 receptors was almost completely confined in the (2*S*, 3*S*) enantiomer (i.e., GR203040), while the corresponding enan-

tiomer (2*R*, 3*R*) (or GR205608) was about 10,000-fold weaker at the human-type NK_1 receptor (Beattie et al. 1995). A significant improvement in the pharmacology of GR203040, over CP96345 and CP99994, was the reduced/lack of affinity of the Glaxo compound for various Na^+ or Ca^{2+} ion channels; a side-effect that had seriously hampered the use of both Pfizer compounds (Beattie et al. 1995). On isolated tissues GR203040 potently, but insurmountably, antagonized NK_1 receptor-mediated responses, probably due to its slow dissociation rate from the receptor (Table 3). In vivo, GR203040 was found to gain rapid access to the CNS (in the gerbil) and to reduce carotid vascular resistance (induced by local administration of SP methyl ester; Table 6); this latter effect was thought to be representative of NK_1 receptor-mediated craniovascular dilatation. Moreover, GR203040 antagonized NK_1 receptor-mediated cranial vasodilatation in vitro (in dog cerebral arteries) and plasma protein extravasation in the rat dura mater; these results prompted Beattie and coworkers (1995) to propose GR203040 as a candidate therapeutic agent in the clinical management of migraine. In addition, GR203040 was shown to possess potent (about 30-fold more than CP99994), prolonged and broad-spectrum antiemetic activity in the ferret, dog and *Suncus murinus*, after intravenous or oral administration (Table 6), suggesting that it could prove useful in the control of emesis associated with cancer chemotherapy and possibly with other diseases (Ward et al. 1995; Gardner et al. 1995).

Further structure–activity studies on close analogs of GR203040, aimed at the optimization of the antiemetic properties of this structural type of molecule, led to the identification of GR205171, a trifluoromethyl-substituted compound at the C-1 position of the tetrazole ring present in the GR203040 structure (Gardner et al. 1996; Fig. 2). GR205171 was shown to possess a pattern of affinities for tachykinin and nontachykinin receptors very similar to that shown by GR203040, with a potency at various ion channels being at least 1000-fold lower than that at human NK_1 receptors (Gardner et al. 1996; Table 5). GR205171 was reported to produce long-lasting antiemetic activity in the ferret, dog and *Suncus murinus*, against a variety of emetic stimuli, including stimuli (like morphine, copper sulfate and motion) that produce emetic responses refractory to treatment with 5-HT_3 receptor antagonists, at doses approximately threefold lower than those required with GR203040 (Gardner et al. 1996). In particular GR205171 was fully active in both ferret and dog following oral administration at doses only threefold higher than the minimum fully effective parenteral dose (Gardner et al. 1996; Table 6). Subsequent investigations have suggested a potential usefulness of GR205171 in counteracting drug-induced conditioned aversive behavior and nausea, as shown by McAllister and Pratt (1998) who found GR205171 capable of preventing conditioned taste aversions in rats provoked by administration of apomorphine or amphetamine. In clinical trials, GR205171 has been proven effective in reducing the rate of postoperative nausea and vomiting in patients undergoing major gynecological surgery, being well tolerated and producing no major adverse effect (Diemunsch et al. 1999). In contrast, GR205171 was found ineffective in preventing motion-induced nausea in 16

healthy subjects, even in combination with the 5-HT_3 receptor antagonist ondansetron (Reid et al. 2000).

4.5
L754030 (MK869) and Related Compounds

Structure–activity studies aimed at obtaining highly potent and selective NK_1 receptor antagonists, devoid of the side effects accompanying the first Pfizer compounds, were undertaken at Merck, based on the backbone of CP96345 first, and of CP99994 later. A first class of Merck compounds derived in such way were quinuclidine benzylether derivatives; one of the most important representative was a bis-trifluoromethyl analog, named L709210 (Fig. 2) whose affinity for human NK_1 receptors ranged from 0.7 nM to 1.3 nM, depending on the diastereoisomer under examination (Swain et al. 1993). Another series of early Merck compounds is that of benzylamino piperidines. The first example of these structures is L733060 (Fig. 2), which bears a 3,5-bistrifluoromethyl benzylether moiety in the place of the 2-methoxy benzylamine moiety present in CP99994 (Harrison et al. 1994). L733060 was shown to be a stereoselective (2S, 3S being the active enantiomer) highly potent ligand of human NK_1 receptors in vitro (Harrison et al. 1994; Seabrook et al. 1996; Table 5) and a potent antagonist of NK_1 receptor-mediated neurogenic plasma protein extravasation in rats (Seabrook et al. 1996). L733060 also reduces the late phase of the nociceptive response (paw licking) to formalin injection in gerbils [50% inhibitory dose IC_{50}) of about 0.2 mg/kg i.v.] (Rupniak et al. 1996). L 733060 has been instrumental in demonstrating an antidepressant-like activity of the tachykinin NK_1 receptor antagonists. In preclinical assays, Kramer and coworkers (1998) showed that L733060 (but not its less active enantiomer L733061) abolished vocalizations elicited in guinea pigs by intracerebroventricular (i.c.v.) injection of an NK_1 receptor-selective agonist, an effect shared by the antidepressants imipramine and fluoxetine. Furthermore, L733060 was able to prevent vocalizations evoked in guinea pig pups by transient maternal separation, as did certain antidepressant or anxiolytic drugs (Kramer et al. 1998). This study provided the first preclinical evidence that blockade of central NK_1 receptors could inhibit a psychological stress response, and led Merck researchers to a undertake clinical trials to evaluate MK869 (see below) in patients suffering from depression associated with anxiety.

Subsequent structure–activity efforts on this series of compounds were devoted to reducing the affinity–typical of such structures–for Ca^{2+} channels and improving oral bioavailability (for a chemical review of these structures see Quartara and Maggi 1997). One of the most promising outcomes was L742694 (Fig. 2), a compound bearing morpholine at the place of piperidine in the precursor L733060 and a triazolone structure attached to the morpholine nitrogen (Hale et al. 1996). As compared to CP99994, L742694 gained affinity at human NK_1 receptors (Table 5) while showing threefold lower affinity than CP99994 for L-type Ca^{2+} channels (Hale et al. 1996). Further investigation into the molecular

mechanism of interaction of L742694 with the human NK_1 receptor, in which the radiolabeled [^{3}H]L742694 was used (Cascieri et al. 1997), demonstrated that it behaves as a competitive antagonist but, due to its slow rate of dissociation from the receptor, can act as a pseudoirreversible antagonist under particular experimental conditions. In vivo, L742694, given orally, inhibited SP-induced plasma protein extravasation in guinea pig skin with about 170-fold higher potency than CP99994 (ID_{50}=0.009 vs. 1.6 mg/kg) (Hale et al. 1996), and blocked acute retching induced by cisplatin in ferrets (Rupniak et al. 1997). Structural modifications of L742694 were introduced to reduce metabolic degradation of this morpholine acetal derivative. The result of these efforts was the discovery of L754030 (also known as aprepitant or MK869; Fig. 2) a fluorine derivative of L742694, showing conserved high affinity for the human NK_1 receptor (Table 5) and high oral efficacy in preventing vascular leakage induced by resiniferatoxin in guinea pigs or foot tapping behavior induced by central infusion of an NK_1 selective agonist (GR73632) in gerbils (Hale et al. 1998). In particular, antagonist administration 24 h prior to resiniferatoxin challenge or GR73632 infusion revealed a three- to tenfold higher potency of L754030 than L742694, whereas antagonists like CP122721 (Sect. 4.1) or GR205171 (Sect. 4.4), although being equipotent/more potent than L754030 within 1h of administration, were ineffective or tenfold less effective than L754030 24 h post-treatment, demonstrating a very long duration of action of L754030 relative to piperidine-based compounds (Hale et al. 1998). In addition, L754030 proved to be more potent than L742694 in preventing cisplatin-, morphine- or apomorphine-induced emesis in ferrets; and was selected by Merck as candidate for treatment of various pathological conditions in which NK_1 receptors are thought to play a role (Hale et al. 1998). As a matter of fact, L754030 (300 mg/day) was the first tachykinin NK_1 receptor antagonist to be shown efficacious in producing antidepressant and anxiolytic activity in outpatients with major depressive disorder and high anxiety levels, in a randomized, double-blind, placebo-controlled study (Kramer et al. 1998). Concomitant biochemical investigations in gerbils led Kramer and coworkers (1998) to claim that L754030 works differently from established antidepressant drugs, with no augmentation of norepinephrine or serotonin function provoked by L754030 administration. Subsequently, L754030 and its water-soluble prodrug, L758298 (Hale et al. 2000) were found effective in reducing delayed emesis induced by cisplatin treatment in patients with malignant disease (Navari et al. 1999; Van Belle et al. 2002). L754030 (given as dual therapy with dexamethasone) was also effective in reducing acute emesis, although dual therapy with ondansetron and dexamethasone was superior in this early phase (van Belle et al. 2002). A higher efficacy of L754030 in controlling delayed vs. acute emesis was further supported by a study performed on 53 cisplatin-naive patients who received 60–100 mg of the L754030 prodrug, L758298 (Cocquyt et al. 2001). Owing to its effectiveness in preventing chemotherapy-induced nausea/emesis, L754030 (Emend^R) has recently been approved in the USA for the treatment of this pathological condition. Thus, L754030 is the first compound arising from the research efforts in the tachykinin field to become a drug for the manage-

ment of human diseases. L754030 underwent further clinical trials to assess its efficacy in the control of painful conditions such as dental pain or osteoarthritic neuropathic pain. However, doses [300 mg per os (p.o.)] of L754030 found effective in the treatment of depression or emesis failed to be analgesic in both painful conditions (Rupniak and Kramer 1999).

Several chemical analogs of L754030 have been synthesized by Merck, and proven effective as antidepressant/antiemetic agents in preclinical animal models. One of the most relevant is L760735 which is able to prevent vocalizations evoked in guinea pig pups by transient maternal separation (Kramer et al. 1998) and to increase the time spent by gerbils in social interaction (Cheeta et al. 2001); this latter study suggests that this compound could be useful in anxiolytic therapy.

A different class of NK_1 receptor antagonists, derived from the screening of a Merck sample collection, is represented by N-ethyl-L-tryptophan benzyl esters, whose most representative and active compound is L732138 (Fig. 2; MacLeod et al. 1994). L732138 possesses nanomolar affinity for human NK_1 receptors (Table 5) and a 200-fold reduced affinity for the rat-type NK_1 receptor (Cascieri et al. 1994). Intensive work was undertaken by Merck chemists on the L732138 backbone, in order to minimize its rapid metabolic degradation and improve both solubility and bioavailability. Their efforts led to the synthesis of L737488 (Fig. 2), a compound showing higher affinity than L732138 at human NK_1 receptors (Table 5) along with good oral activity in reducing SP-induced plasma protein extravasation in guinea pigs (ID_{50}=1.8 mg/kg, p.o.) and improved solubility in water (MacLeod et al. 1995).

4.6
LY 303870 and Related Compounds

A new class of NK_1 receptor antagonists was developed at Lilly, by optimization of N-acetylated tryptophan amides and esters (Hipskind et al. 1996). The most interesting compound of this series is LY 303870 (lanepitant), in which the carboxy group of tryptophan is reduced and functionalized with an *N*-acetyl tertiary amide (Fig. 3). LY 303870 showed subnanomolar affinity for human NK_1 peripheral and central receptors, approximately 50-fold lower affinity for rat or mouse NK_1 receptors, and very low affinity for tachykinin NK_2 or NK_3 receptors, T, L and N-type calcium channels (p*Ki* values lower than 6.0 at these latter receptors) (Table 5; Gitter et al. 1995). The ability of LY303870 to interact with NK_1 receptors was highly stereoselective, as demonstrated by its (*S*)-enantiomer (named LY306155) which bound to human or animal NK_1 receptors with 1,000–15,000-fold lower affinities (Gitter et al. 1995). LY303870 proved to be a very potent antagonist of NK_1-receptor mediated effects in both the rabbit isolated vena cava (Table 3) and in vivo experiments, in which it potently inhibited both bronchoconstriction (ID_{50}=13μg/kg i.v.) and pulmonary microvascular leakage (ID_{50}=8μg/kg i.v.) induced by [Sar^9]SP sulfone in the guinea pig (Gitter et al. 1995). Subsequently, the antinociceptive potential of LY303870 was examined in

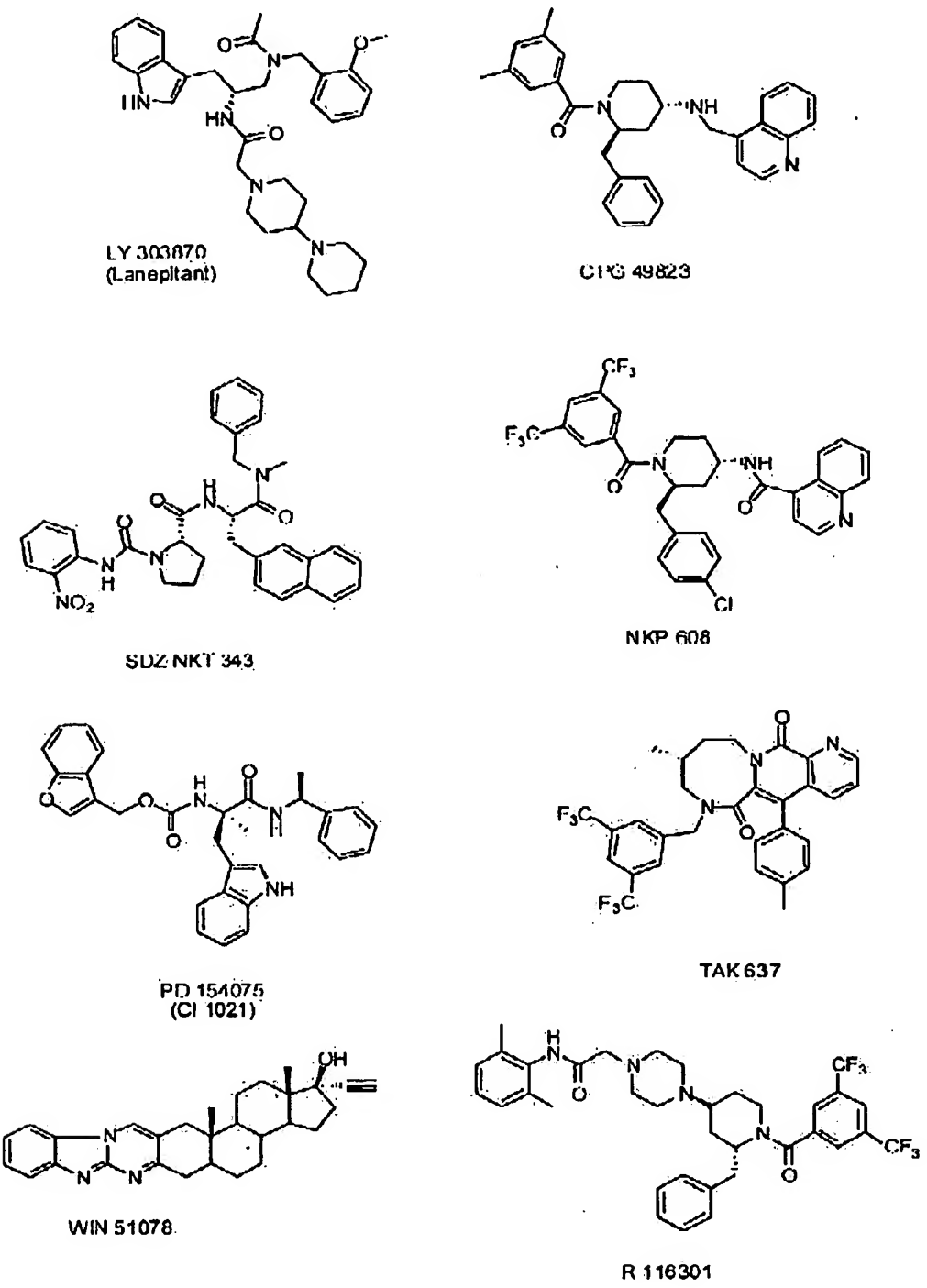

Fig. 3 Chemical structures of nonpeptide tachykinin NK_1 receptor antagonists: III

typical tests of pain such as the tail flick latency test and the formalin test in rats (Iyengar et al. 1997). In both bioassays LY303870 was shown to produce long-lasting antinociceptive effects after systemic or oral administration, in a range of quite high doses: 1–30 mg/kg (Iyengar et al. 1997). Lower doses of LY303870

(1–100μg/kg) were required to prevent plasma protein extravasation induced by electrical stimulation of the trigeminal ganglion in guinea pigs, a model thought to be representative of migraine of neurogenic origin (Phebus et al. 1997). Nevertheless, subsequent clinical trials with LY303870 provided disappointing results, as it was found ineffective in relieving osteoarthritic pain (Goldstein et al. 2000), in preventing migraine (Goldstein et al. 2001a) and in relieving pain of diabetic neuropathy (Goldstein et al. 2001b). The failure of LY303870 as an analgesic in the clinical trials was first attributed to its inadequate penetration of the blood–brain barrier (Goldstein et al. 2000), a possibility that would explain its low potency in preventing foot-tapping in gerbils and cisplatin-induced emesis in ferrets (Table 4; Rupniak et al. 1997). In this regard, Urban and Fox (2000) have argued that the failure of LY303870 to exert analgesic effects is not surprising, since this compound is poorly effective even in inflammatory and neuropathic models of the guinea pig, a species endowed with a human-like NK_1 receptor, and for this reason more predictive than other models of pain in rodents. Whatever the explanation, it is worth mentioning that other trials with compounds better at penetrating the CNS (such as L754030, see Sect. 4.5) did not provide pain relief either. More recently, the effectiveness of LY303870 in a mouse model of inflammatory bowel disease has been reported, both in reducing the severity of IBD and in allowing the partial healing of intestinal lesions in mice with preexisting IBD (Sonea et al. 2002; Table 6).

4.7
CGP49823; SDZNKT343; NKP608

A new class of piperidine-based antagonists was obtained at Ciba-Geigy, through the modification of a lead derived from the screening of a chemical collection. One of the most interesting compounds of this series derived from lead optimization is CGP49823 (Fig. 3; Hauser et al. 1994). CGP49823 showed good affinity for bovine NK_1 receptors (Table 5) and antagonized with similar affinity (IC_{50}=13 nM) SP-induced inositol monophosphate production in human U-373MG cells and SP-induced contractions in the rabbit vena cava (Table 3), whereas it was approximately 100-fold weaker at NK_2 or NK_3 receptors (Table 5; Hauser et al. 1994). In vivo, CGP49823 increased active social time in rats and reduced the immobility time of rats in the swim test, after both single or subchronic oral administration of high doses (3–30 mg/kg) (Vassout et al. 1994). These results led the authors to indicate a potential anxiolytic and antidepressant effect of CGP49823 (Table 6). High doses of CGP49823 were also required to inhibit thumping behavior induced by i.c.v. administration of SP methylester in gerbils (ED_{50}=50 mg/kg p.o.) (Vassout et al. 1994). This result was confirmed by Rupniak and coworkers (1997) who, in addition, found CGP49823 to be ineffective in preventing cisplatin-induced acute retching in ferrets and concluded that this compound does not readily cross the blood–brain barrier. On this basis, Rupniak and Kramer (1999) questioned whether the reported antidepressant

effects produced by CGP49823 in rats are attributable to antagonism of NK_1 receptor rather than another pharmacological effect.

Another series of compounds discovered at Novartis was obtained by combination of a series of 2-halo-substituted benzylthioureas with aromatic amino acid amides leading to a series of 2-NO_2 phenylthiourea analogs, whose most active derivative was SDZNKT343 (Fig. 3; Walpole et al. 1998b). SDZNKT343 showed subnanomolar affinity for human NK_1 receptors (Table 5) and at least 500-fold lower affinity for the rat NK_1 receptor, whereas its less active (*R*,*R*)-enantiomer was about 1,000 times less active at all receptors (Walpole et al. 1998a). The mechanism of interaction of SDZNKT343 with human NK_1 receptors was clearly noncompetitive, as was the antagonism by SDZNKT343 of [Sar^9]SP sulfone-induced contractions in the guinea pig ileum (Walpole et al. 1998a). SDZNKT343 is an extremely selective ligand of human NK_1 receptors relative to a wide array of binding sites: however, at 10μM it inhibited voltage-activated Ca^{2+} and Na^+ currents in guinea pig dorsal root ganglion neurons, as did CP96345 and CP99994 (Walpole et al. 1998a). In vivo, SDZNKT343 (30 mg/kg p.o.) reduced mechanical hyperalgesia elicited by intraplantar carrageenan in guinea pigs, being significantly more potent than other NK_1 receptor antagonists like RPR100893 or SR140333, whereas it was less efficacious in reducing thermal hyperalgesia in carrageenan-pretreated guinea pigs (Campbell et al. 2000). In addition, SDZNKT343 (30 mg/kg p.o.) reversed by 60% the plasma protein extravasation induced by Freund's complete adjuvant in the guinea pig knee joint. On the basis of these results there is great expectation that SDZNKT343 could exert analgesic activity in man, as proposed by Urban and Fox (2000) who outlined the high reliability of the guinea pig models of neuropathic pain and inflammation over similar models in rodents.

More recently Novartis has presented a novel 4-amino piperidine derivative: NKP608 (Fig. 3; Vassout et al. 2000). In vitro, NKP608 showed nanomolar affinity for bovine NK_1 receptors (Table 5), and blocked with similar affinity (IC_{50}=2.6 nM) SP-induced inositol monophosphate production in human U-373MG cells, while it bound with only tenfold reduced affinity to the rat NK_1 receptor (Vassout et al. 2000). In in vivo behavioral tests, such as the social interaction test, oral doses of NKP608 specifically increased the time spent by rats in social contact, and in a social exploration test similar doses of NKP608 increased the time spent by the intruder rat in social contact with the resident rat. Both effects of NKP608 resembled those exerted by the benzodiazepine drug chlordiazepoxide, and suggested a possible anxiolytic use of NKP608 in humans (Table 6; Vassout et al. 2000). In the hind foot thumping model in gerbils, NKP608 was a highly effective and long-lasting antagonist following oral administration of doses comparable to those found effective in the rat: this finding provided an indirect demonstration of a good ability of NKP608 to cross the blood–brain barrier (Vassout et al. 2000). Anxiolytic-like effects of NKP608 have recently been reported also in gerbils, a species bearing a NK_1 receptor more similar to the human type than the rat. In the gerbil model, low doses of NKP608 given orally were reported to be as effective as the anxiolytic drug

chlordiazepoxide (Gentsch et al. 2002). In addition to its anxiolytic activity, NKP608 has been reported to produce antidepressant-like effects in a chronic mild stress model of depression in rats (Papp et al. 2000). In this latter model NKP608 (0.03–0.1 mg/kg p.o.) produced effects comparable to those of imipramine, but the onset of action was faster with NKP608 than with the tricyclic antidepressant. Interestingly, the dose–response curves for both the anxiolytic (Vassout et al. 2000) and antidepressant (Papp et al. 2000) effects of NKP608 in rats were bell-shaped: i.e., the positive effect seen at relatively low doses was fading out at higher doses. The cause of this behavior of NKP608 is not fully understood (see Vassout et al. 2000, for tentative explanations).

4.8
PD154075 (CI1021)

A new series of compounds containing a tryptophan motif was developed at Parke-Davis in the mid-1990s, starting from a dipeptide library, from which a lead peptide compound (Z-Trp-Phe-NH_2) showing micromolar affinity at tachykinin receptors was obtained (Boyle et al. 1994). Further modifications of this lead yielded PD154075 (or CI1021), a compound bearing a methyl-tryptophan group and a 2-benzofuran moiety (Fig. 3). PD154075 showed high affinity for NK_1 receptors of man, guinea pig and other human-related species in binding and functional assays (Tables 3 and 5), whereas it bound with approximately 300-fold lower affinity to rat or mouse NK_1 receptors (Boyle et al. 1994; Singh et al. 1997). In vivo, PD154075 potently (ID_{50}=0.02 mg/kg i.v.) blocked SP methylester-induced plasma protein extravasation in the guinea pig bladder (Boyle et al. 1994) and dose-dependently [1–100 mg/kg subcutaneously (s.c.)] antagonized [Sar^9]SP sulfone-induced foot tapping in the gerbil, this latter effect supporting a central mechanism of action for this compound (Singh et al. 1997). Brain penetration by PD154075 after oral administration in rats was confirmed by extraction and HPLC assay: its absolute oral bioavailability in this species was 49±15% (Singh et al. 1997). An antiemetic potential of PD154075 was shown in the ferret: in this species PD154075 (10 mg/kg s.c. three times a day for 3 days) almost completely blocked both the acute and delayed emetic response to cisplatin (Singh et al. 1997). In a model of postoperative pain in the rat, PD154075–given before, not after surgery–selectively blocked both mechanical and thermal hypersensitivity of the operated rats and, unlike morphine, did not modify the length of anesthetic-induced sleep (Gonzalez et al. 1998; Table 6). The analgesic activity of PD154075 has further been shown in a chronic constrictive injury model (sciatic nerve ligation), in which rats developed thermal and mechanical hyperalgesia along with cold, dynamic and static allodynia (Gonzalez et al. 2000). PD154075 blocked all these responses (except dynamic allodynia), without inducing tolerance during a 10-day long treatment with 100 mg/kg/day s.c. Importantly, PD154075 blocked hypersensitivity in guinea pigs induced by sciatic nerve ligation, with a minimum effective dose of 0.1 mg/kg p.o.; on the basis of these re-

sults it has been proposed as a possible therapeutic agent in inflammatory and neuropathic pain (Gonzalez et al. 2000).

4.9 TAK637

In 1995 Natsugari and coworkers at Takeda presented a new series of N-benzylcarboxyamide compounds, that were the result of optimization of a benzodiazepine-based cholecystokinin antagonist chosen as the lead compound. The most active compound of this series showed subnanomolar affinity for the human NK_1 receptor along with high oral potency in preventing capsaicin-induced plasma protein extravasation in guinea pigs (Natsugari et al. 1995). Further optimization of this series of compounds yielded TAK637 (Fig. 3), a very potent antagonist of the human NK_1 receptor, with >100-fold reduced potency at the rat NK_1 receptor (Natsugari et al. 1999; Table 5). Moreover, TAK637 has been claimed to be at least 2000 times weaker at NK_2 or NK_3 than human NK_1 receptors (Okano et al. 2002). However, it should be noted that while the affinity of TAK637 for NK_3 receptors (pK_i <6.0) has been checked in a binding assay on guinea pig cerebral cortex membranes, that for NK_2 receptors (pA_2=6.0) has been evaluated in functional experiments on the guinea pig ileum, using NKA as the agonist (Natsugari et al. 1999). Thus, both the presence of additional NK_1 and NK_3 receptors in the tissue, and the poor selectivity of the agonist used (NKA) make the outcome of the latter experiments questionable. More recently, Venkova and coworkers (2002) have established that in the guinea pig isolated colon the ratio between antagonist potencies of TAK637 at NK_1 vs. NK_2 receptors is at least 20–50:1; however concentrations of TAK637 higher than 0.1μM were not tested in that study, so that determination of an exact potency ratio was hampered (Table 3). In vivo, systemic TAK637 increased the volume threshold of saline required to elicit the micturition reflex in anesthetized guinea pigs without decreasing the voiding pressure; an effect that was reproduced in unanesthetized animals after oral administration of low doses of TAK637 (Doi et al. 1999). Furthermore, TAK637 was able to decrease the number, but not amplitude, of rhythmic bladder contractions elicited by urinary bladder wall distension (by saline), and also dose-dependently reduced the number of animals responding with micturition to topical application of capsaicin onto the surface of the bladder dome (Doi et al. 2000). These results suggest TK637 as possible drug candidate for the control of urinary incontinence due to detrusor overactivity (Doi et al. 1999, 2000; Table 6)

In a model of intestinal transit in the gerbil, TK637 selectively reduced the increase of fecal pellet output provoked by both administration of an NK_1 receptor-selective agonist or by restraint stress, without modifying spontaneous excretion of fecal pellets in control animals (Okano et al. 2001). In a model of visceral pain, intraduodenal TK637 stereoselectively reduced the number of abdominal contractions provoked by colorectal distension in rabbits previously subjected to colonic irritation (Okano et al. 2002). Intrathecal administration of

TK637 was also effective in this model (Okano et al. 2002), thus showing that central (spinal cord located) receptors are the most probable target of the action of TK637. These results led Okano and coworkers (2001, 2002) to suggest that TK637 may be useful in treating functional bowel disorders such as IBS.

4.10
Other Nonpeptide Compounds

WIN51078 (Fig. 3) is one of the most interesting heterosteroid compounds of a series obtained through the screening of natural products, being endowed with appreciable affinity for the rat NK_1 receptor (IC_{50}=50 nM in rat forebrain membranes) (Venepalli et al. 1992). However, WIN51078 possesses a dramatically lower (about 400-fold) affinity for the human NK_1 receptor as compared to the rat type (Sachais and Krause 1994). This characteristic of action, while reinforcing the concept that important species-related differences exist among tachykinin receptors, has represented a serious drawback that hampered the development of WIN51078 and related compounds into drugs to be used in human diseases.

R116301 (Fig. 3) has recently been introduced by Johnson & Johnson as a potent and selective piperidine-based NK_1 receptor antagonist (Megens et al. 2002). R116301 is endowed with subnanomolar affinity for human NK_1 receptors and over 200-fold selectivity relative to human NK_2, NK_3 and rat NK_1 receptors (Table 5). In vivo, R116301 has been shown to potently antagonize both peripheral (SP-induced plasma protein extravasation) and central (thumping in gerbils) NK_1 receptor-mediated effects. In addition, R116301 is able to reduce/prevent emesis caused by various emetic stimuli in ferrets, cats and dogs, also after oral pretreatment (Megens et al. 2002). The ratio of oral vs. parenteral activity ranges from 0.2 to 2.7 in the species examined, and the action lasts from 6.5 to 16 h. An oral dose of 300 mg R116301 was found effective in reducing SP-induced dilation of the precontracted hand vein in human volunteers (Romerio et al. 1999). The available results obtained with R116301 make it a promising tool to be exploited in clinical trials for various human diseases involving a role of NK_1 receptors.

5
Therapeutic Perspectives for NK_1 Receptor Antagonists

Historically, the first indication for which a tachykinin NK_1 receptor antagonist was expected to be efficacious was pain, as the early evidence for an involvement of SP in pain transmission and perception had been collected since the 1950s, and subsequently this concept had been widely supported by a plethora of preclinical studies (for a review see Quartara and Maggi 1998). Thus, it was not surprising to see the first potent and selective nonpeptide antagonists such as CP96345 (Sect. 4.1), RP67580 (Sect. 4.2) and SR140333 (Sect. 4.3) to produce antinociceptive and antiinflammatory effects in classical animal models for test-

ing analgesic drugs, thereby providing further support for a role of NK_1 receptors in nociception and inflammation (Table 6). Nevertheless, after promising results had been obtained with CP99994 in patients suffering with postoperative dental pain (Sect. 4.1), all subsequent clinical trials with CP99994 itself and several other nonpeptide compounds such as LY303870 (Sect. 4.6), L754030 (or MK869; Sect. 4.5) and RPR100893 (Sect. 4.2) were disappointing, as they failed to demonstrate analgesic effects in patients affected by osteoarthritis, neuropathic pain, dental pain and migraine. Several explanations have been put forward in an attempt to explain this discrepancy between preclinical and clinical results (Rupniak and Kramer 1999; Hill 2000a, 2000b; Urban and Fox 2000). One of the criticisms was that certain antagonists (like LY303870 and others) are ineffective as analgesics in clinical trials because of an inadequate penetration of the blood–brain barrier. However, it is worth noting that better CNS penetrating compounds (like L754030) did not provide pain relief either (Rupniak and Kramer 1999).

At present, there are two main pathological conditions in which tachykinin NK_1 receptor-selective antagonists have been confirmed effective in humans: emesis, and affective disorders such as depression and anxiety. The first evidence that an NK_1 antagonist could be useful in the control of emesis provoked by cisplatin and various other stimuli, was collected 10 years ago with CP99994 in the ferret (Sect. 4.1). Subsequently, CP99994 and other antagonist compounds like GR203040, GR205171 (Sect. 4.4), PD154075 (Sect. 4.8) and others have proved effective in blocking the effects of various emetogens in animal species such as *Suncus murinus*, dog and cat. In man, CP122721 has been the first compound reported successful in reducing delayed emesis in patients under chemotherapy (Sect. 4.1). Since it was less effective in the control of nausea it was discontinued for this indication (Evangelista 2001). Another compound shown to be effective in the control of chemotherapy-induced emesis in humans is L754030 (aprepitant, Emend^R; Sect. 4.5) that has recently been approved in the USA for the combination treatment of this pathological condition. Thus, L754030 is the first compound arising from research in the field of tachykinins to become a drug for the care of human diseases. GR205171 has also been proven efficacious in reducing postoperative nausea and emesis, but unable to reduce motion-induced nausea in healthy volunteers (Sect. 4.4).

CGP49823 (Sect. 4.7) was shown in 1994 to be active in the social interaction test and swimming test in rats, and on the basis of these results it was claimed to possess a potential anxiolytic/antidepressant property. The efficacy of CGP49823 in these tests has been questioned afterwards, because of the poor ability shown by this compound to cross the blood–brain barrier (Rupniak and Kramer 1999). Demonstration of antidepressant and possibly anxiolytic activity afforded by an NK_1 receptor antagonist has been furnished by Kramer and coworkers (1998), in both animals and humans, by the use of L754030 (MK869) and related compounds (Sect. 4.5). NKP608 is another promising compound in this area, having been shown effective in affording anxiolytic-like effects in rats and gerbils and antidepressant effects in rats (Sect. 4.7; Table 6). Tachykinin

NK_1 receptor-selective antagonists are also expected to provide therapeutic effects in peripheral inflammatory diseases in which the role of both tachykinins and NK_1 receptors have been clearly documented in preclinical investigations. The main peripheral indications are: asthma/bronchial hyperreactivity and inflammatory bowel disease. A possible antiasthmatic activity of these compounds was envisaged with the first peptidic antagonists such as FK888 or MEN11467 (Sect. 3.2) which were found effective in preventing both bronchoconstriction and plasma protein extravasation in the airways, induced by tachykinins and/or other stimuli. However, the clinical trials performed with FK888 and CP99994 (Sect. 4.1) have provided negative results. Now there is general expectation that mixed NK_1/NK_2 receptor antagonists might afford beneficial effects in asthma, relative to NK_1 or NK_2 receptor-selective compounds. Tachykinins are thought to be important mediators involved in the genesis/maintenance of various inflammatory gastrointestinal diseases, a concept that is based on an increasing number of studies that have been reviewed extensively elsewhere (e.g., Holzer and Holzer-Petsche 1997; Holzer 1998; Bueno 2000). Various NK_1 receptor antagonists have been found effective in reducing the severity of experimental IBD in animals, including SR140333 (Sect. 4.3), LY303870 (Sect. 4.6) and TAK637 (Sect. 4.9; Table 6). This latter compound has been found effective in a model of visceral pain in rabbits and on this basis it has been proposed for the treatment of IBS. However, its selectivity for NK_1 over NK_2 receptors is not well documented, and the possibility that reduction of visceral pain by TAK637 stems from blockade of NK_2 receptors remains to be investigated. Nevertheless, the effectiveness of a very selective NK_1 receptor antagonist, such as CJ11974 (ezlopitant), in patients affected by IBShas been documented recently (Sect. 4.1).

Acknowledgements. The authors are grateful to Dr. Laura Quartara, Chemical Department, Menarini Ricerche, Florence, Italy, for drawing the chemical structures of the non-peptide compounds and for critically reading the text.

References

Amann R, Schuligoi R, Holzer P, Donnerer J (1995) The non-peptide NK_1 receptor antagonist SR140333 produces long-lasting inhibition of neurogenic inflammation, but does not influence acute chemo- or thermonociception in rats. Naunyn Schmiedebergs Arch Pharmacol 352:201–205

Aramori I, Morikawa N, Zenkoh J, O'Donnell N, Iwami M, Kojo H, Notsu Y, Okuhara M, Ono S, Nakanishi S (1994) Subtype- and species-selectivity of a tachykinin receptor antagonist, FK888, for cloned rat and humantachykinin receptors. Eur J Pharmacol 269:277–281

Astolfi M, Patacchini R, Maggi M, Manzini S (1997) Improved discriminatory properties between human and murine tachykinin NK_1 receptors of MEN 10930: a new potent and competitive antagonist. Neuropeptides 31:373–379

Barr AJ, Watson SP (1993) Non-peptide antagonists CP 96,345 and RP 67,580, distinguish species variants in tachykinin NK-1 receptors. Br J Pharmacol 108:223–227

Beattie DT, Beresford IJ, Connor HE, Marshall FH, Hawcock AB, Hagan RM, Bowers J, Birch PJ, Ward P (1995) The pharmacology of GR203040, a novel, potent and selective non-peptide tachykinin NK_1 receptor antagonist. Br J Pharmacol 116:3149–3157

Beaujouan JC, Heuillet E, Petitet F, Saffroy M, Torrens Y, Glowinski J (1993) Higher potency of RP 67580, in the mouse and the rat compared with other nonpeptide and peptide tachykinin NK_1 antagonists. Br J Pharmacol 108:793–800

Beresford IJ, Birch PJ, Basalt M-C, Rogers H, Fernandez L, Hagan RM (1991a) Effect of the spirolactam NK-1 receptor antagonist GR 82334 on neurokinin-1 and electrical stimulation-induced oedema in the rat. Br J Pharmacol 102:360P

Beresford IJ, Birch PJ, Hagan RM, Ireland SJ (1991b) Investigation into species variants in tachykinin NK_1 receptors by use of the non-peptide antagonist, CP-96,345. Br J Pharmacol 104:292–293

Birch PJ, Harrison SM, Hayes AG, Rogers H, Tyers MB (1992) The non-peptide NK_1 receptor antagonist, (+/-)-CP-96,345, produces antinociceptive and anti-oedema effects in the rat. Br J Pharmacol 105:508–510

Boni P, Maggi CA, Evangelista S (1994) In vivo evidence for the activation of a septide-sensitive tachykinin receptor in guinea-pig bronchoconstriction. Life Sci 54:PL327–PL332

Bonnet J, Kucharczyk N, Robineau P, Lonchampt M, Ducquet C, Regoli D, Fauchere JL, Canet E A (1996) Water-soluble, stable dipeptide NK_1 receptor-selective neurokinin receptor antagonist with potent in vivo pharmacological effects: S18523. Eur J Pharmacol 310:37–46

Bountra C, Bunce K, Dale T, Gardner C, Jordan C, Twissell D, Ward P (1993) Anti-emetic profile of a non-peptide neurokinin NK_1 receptor antagonist, CP-99,994, in ferrets. Eur J Pharmacol 249:R3–R4

Boyle S, Guard S, Higginbottom M, Horwell DC, Howson W, McKnight AT, Martin K, Pritchard MC, O'Toole J, Raphy J (1994) Rational design of high affinity tachykinin NK_1 receptor antagonists. Bioorg Med Chem 2:357–370

Bremer AA, Tansky FM, Wu M, Boyd ND, Leeman S (2001) Direct evidence for the interaction of neurokinin A with the tachykinin NK_1 receptor in tissue. Eur J Pharmacol 423:143–147

Bueno L, Fioramonti J, Garcia-Villar R (2000) Pathobiology of visceral pain: molecular mechanisms and therapeutic implications III Visceral afferent pathways: a source of new therapeutic targets for abdominal pain. Am J Physiol 278:G670–G676

Caesar M, Scabrook GR, Kemp JA (1993) Block of voltage-dependent sodium currents by the substance P receptor antagonist (±)CP 96,345 in neurons cultured from the rat cortex. J Physiol 459:397P

Campbell EA, Gentry C, Patel S, Kidd B, Cruwys S, Fox AJ, Urban L (2000) Oral anti-hyperalgesic and anti-inflammatory activity of NK_1 receptor antagonists in models of inflammatory hyperalgesia of the guinea-pig. Pain 87:253–263

Cascieri MA, Macleod AM, Underwood D, Shiao LL, Ber E, Sadowski S, Yu H, Merchant KJ, Swain CJ, Strader CD, Fong TM (1994) Characterization of the interaction of N-acyl-L-tryptophan benzyl ester neurokinin antagonists with the human neurokinin-1 receptor. J Biol Chem 269:6587–6591

Cascieri MA, Ber E, Fong TM, Hale JJ, Tang F, Shiao LL, Mills SG, MacCoss M, Sadowski S, Tota MR, Strader CD (1997) Characterization of the binding and activity of a high affinity, pseudoirreversible morpholino tachykinin NK_1 receptor antagonist. Eur J Pharmacol 325:253–261

Cellier E, Barbot L, Iyengar S, Couture R (1999) Characterization of central and peripheral effects of septide with the use of five tachykinin NK_1 receptor antagonists in the rat. Br J Pharmacol 127:717–728

Cheeta S, Tucci S, Sandhu J, Williams AR, Rupniak NM, File SE (2001) Anxiolytic actions of the substance P (NK_1) receptor antagonist L-760735 and the 5-HT1A agonist 8-OH-DPAT in the social interaction test in gerbils. Brain Res 915:170–175

Cirillo R, Astolfi M, Conte B, Lopez G, Parlani M, Sacco G, Terracciano R, Fincham CI, Sisto A, Evangelista S, Maggi CA, Manzini S (2001) Pharmacology of MEN 11467: a potent new selective and orally- effective peptidomimetic tachykinin NK_1 receptor antagonist. Neuropeptides 35:137–147

Cocquyt V, Van Belle S, Reinhardt RR, Decramer ML, O'Brien M, Schellens JH, Borms M, Verbeke L, Van Aelst F, De Smet M, Carides AD, Eldridge K, Gertz BJ (2001) Comparison of L-758,298, a prodrug for the selective neurokinin-1 antagonist, L-754,030, with ondansetron for the prevention of cisplatin-induced emesis. Eur J Cancer 37:835–842

Constantine JW, Lebel WS, Woody HA (1991) Inhibition of tachykinin-induced hypotension in dogs by CP-96,345, a selective blocker of NK-1 receptors. Naunyn Schmiedeberg's Arch Pharmacol 344:471–477

Coudore'-Civiale MA, Courteix C, Eschalier A and Fialip J (1998) Effect of tachykinin receptor antagonists in experimental neuropathic pain. Eur J Pharmacol 361:175–184

Courteix C, Lavarenne J, Eschalier A (1993) RP-67580, a specific tachykinin NK_1 receptor antagonist, relieves chronic hyperalgesia in diabetic rats. Eur J Pharmacol 241:267–270

Croci T, Landi M, Emonds-Alt X, Le Fur G, Maffrand JP, Manara L (1997) Role of tachykinins in castor oil diarrhoea in rats. Br J Pharmacol 121:375–380

Cutrer FM, Moussaoui S, Garret C, Moskowitz MA (1995) The non-peptide neurokinin-1 antagonist, RPR 100893, decreases c-fos expression in trigeminal nucleus caudalis following noxious chemical meningeal stimulation. Neuroscience 64:741–750

Delay-Goyet P, Lundberg JM (1991) Cigarette smoke-induced airway oedema is blocked by the NK-1 antagonist, CP 96,345. Eur J Pharmacol 203:157–158

Delay-Goyet P, Franco-Cereceda A, Gonsalves SF, Clingan CA, Lowe JA 3rd, Lundberg JM (1992) CP-96,345 antagonism of NK_1 receptors and smoke-induced protein extravasation in relation to its cardiovascular effects. Eur J Pharmacol 222:213–218

Diemunsch P, Schoeffler P, Bryssine B, Cheli-Muller LE, Lees J, McQuade BA, Spraggs CF (1999) Antiemetic activity of the NK_1 receptor antagonist GR205171 in the treatment of established postoperative nausea and vomiting after major gynaecological surgery. Br J Anaesth 82:274–276

Dionne RA, Max MB, Gordon SM, Parada S, Sang C, Gracely RH, Sethna NF, MacLean DB (1998) The substance P receptor antagonist CP-99,994 reduces acute postoperative pain. Clin Pharmacol Ther 64:562–568

Doi T, Kamo I, Imai S, Okanishi S, Ishimaru T, Ikeura Y, Natsugari H (1999) Effects of TAK-637, a tachykinin receptor antagonist, on lower urinary tract function in the guinea-pig. Eur J Pharmacol 383:297–303

Doi T, Kamo I, Imai S, Okanishi S, Ikeura Y, Natsugari H (2000) Effects of TAK-637, a tachykinin receptor antagonist, on the micturition reflex in guinea-pigs. Eur J Pharmacol 395:241–246

Eglezos A, Giuliani S, Viti G, Maggi CA (1991) Direct evidence that capsaicin-induced plasma protein extravasation is mediated through tachykinin NK_1 receptors. Eur J Pharmacol 209:277–279

Emonds-Alt X, Doutremepuich J, Heaulme M, Neliat G, Santucci V, Steinberg R, Vilain P, Bichon D, Ducoux J, Proietto V, Van Broeck D, Soubrié P, Le Fur G and Breliere, J (1993) In vitro and in vivo biological activities of SR140333, a novel potent non-peptide NK_1 receptor antagonist. Eur J Pharmacol 250:403–413

Emonds-Alt X, Proietto V, Steinberg R, Oury-Donat F, Vige X, Vilain P, Naline E, Daoui S, Advenier C, Le Fur G, Maffrand JP, Soubrie P, Pascal M (2002) SSR240600 [(*R*)-2-(1-[2-[4-[2-[3,5-bis(trifluoromethyl)phenyl]acetyl]-2-(3,4-dichlorophenyl)-2-morpholinyl]ethyl]-4-piperidinyl)-2-methylpropanamide], a centrally active nonpeptide antagonist of the tachykinin neurokinin-1 receptor: I biochemical and pharmacological characterization. J Pharmacol Exp Ther 303:1171–1179

Evangelista S (2001) Eziopitant. Curr Opin Invest Drugs 2:1441–1443

Fahy JV, Wong HH, Geppetti P, Reis JM, Harris SC, Maclean DB, Nadel JA Boushey HA (1995) Effect of an NK_1 receptor antagonist (CP-99,994) on hypertonic saline-induced bronchoconstriction and cough in male asthmatic subjects. Am J Resp Crit Care Med 152:879–884

Fardin V, Carruette A, Menager J, Bock M, Flamand O, Foucault F, Heuillet E, Moussaoui SM, Tabart M, Peyronel JF, Garret C (1994) In vitro pharmacological properties of RPR 100,893, a novel nonpeptide antagonist of the human NK_1 receptor. Neuropeptides 26:34

Folkers K, Hakanson R, Horig J, Xu JC, Leander S (1984) Biological evaluation of substance P antagonists. Br J Pharmacol 83:449–456

Folkers K, Feng DM, Asano N, Håkanson R, Weisenfeld-Hallin Z, Leander S (1990) Spantide II, an effective tachykinin antagonist having high potency and negligible neurotoxicity. Proc Natl Acad Sci USA 87:4833–4835

Fong TM, Yu H, Strader C (1992) Molecular bases for the species selectivity of the NK_1 receptor antagonists CP 96,345 and RP 67,580. J Biol Chem 267:25668–25671

Fong TM, Yu H, Cascieri MA, Underwood D, Swian CJ, Strader CD (1994)The role of Histidine 265 in antagonist binding to the NK_1 receptor. J Biol Chem 269:2728–2732

Fujii T, Murai M, Morimoto H, Maeda Y, Yamaoka M, Hagiwara D, Miyake H, Ikari N, Matsuo M (1992) Pharmacological profile of a high affinity dipeptide NK_1 receptor antagonist, FK888. Br J Pharmacol 107:785–789

Gardner CJ, Twissell DJ, Dale TJ, Gale JD, Jordan CC, Kilpatrick GJ, Bountra C, Ward P (1995) The broad-spectrum anti-emetic activity of the novel non-peptide tachykinin NK_1 receptor antagonist GR203040. Br J Pharmacol 116:3158–3163

Gardner CJ, Armour DR, Beattie DT, Gale JD, Hawcock AB, Kilpatrick GJ, Twissell DJ, Ward P (1996) GR205171: a novel antagonist with high affinity for the tachykinin NK_1 receptor, and potent broad-spectrum anti-emetic activity. Regul Pept 65:45–53

Garret C, Carruette A, Fardin V, Moussaoui S, Peyronel JF, Blanchard JC, Laduron PM (1991) Pharmacological properties of a potent and selective nonpeptide SP antagonist. Proc Natl Acad Sci USA 88:10208–10211

Gentsch C, Cutler M, Vassout A, Veenstra S, Brugger F (2002) Anxiolytic effect of NKP608, a NK_1-receptor antagonist, in the social investigation test in gerbils. Behav Brain Res 133:363–368

Gesztesi Z, Song D, White PF (1998) Comparison of a new NK-1 antagonist (CP-122,721) to ondansetron in the prevention of post-operative nausea and vomiting. Anesth Anal 86:S32

Gesztesi Z, Scuderi PE, White PF, Wright W, Wender RH, D'Angelo R, Black LS, Dalby PL, MacLean D (2000) Substance P (Neurokinin-1) antagonist prevents postoperative vomiting after abdominal hysterectomy procedures. Anesthesiology 93:931–937

Gether U, Lowe JA 3rd, Schwartz TW (1995) Tachykinin non-peptide antagonists: binding domain and molecular mode of action. Biochem Soc Trans 23:96–102

Gitter BD, Waters DC, Bruns RF, Mason NR Nixon JA, Howbert JJ (1991) Species differences in affinities of nonpeptide antagonists for SP receptors. Eur J Pharmacol 197:237–238

Gitter BD, Bruns RF, Howbert JJ, Waters DC, Threlkeld PG, Cox LM, Nixon JA, Lobb KL, Mason NR, Stengel PW, Cockerham SL, Silbaugh SA, Gehlert DR, Schober DA, Iyengar S, Calligaro DO, Regoli D, Hipskind PA (1995) Pharmacological characterization of LY303870: a novel, potent and selective nonpeptide substance P (neurokinin-1) receptor antagonist. J Pharmacol Exp Ther 275:737–744

Goldstein DJ, Wang O, Todd LE, Gitter BD, DeBrota DJ, Iyengar S (2000) Study of the analgesic effect of lanepitant in patients with osteoarthritis pain. Clin Pharmacol Ther 67:419–426

Goldstein DJ, Offen WW, Klein EG, Phebus LA, Hipskind P, Johnson KW, Ryan RE Jr (2001a) Lanepitant, an NK-1 antagonist, in migraine prevention. Cephalalgia 21:102–106

Goldstein DJ, Wang O, Gitter BD, Iyengar S (2001b) Dose-response study of the analgesic effect of lanepitant in patients with painful diabetic neuropathy. Clin Neuropharmacol 24:16–22

Gonsalves S, Watson J, Ashton C (1996) Broad spectrum antiemetic effects of CP-122,721, a tachykinin NK_1 receptor antagonist, in ferrets. Eur J Pharmacol 305:181–185

Gonzalez MI, Field MJ, Holloman EF, Hughes J, Oles RJ, Singh L (1998) Evaluation of PD 154075, a tachykinin NK_1 receptor antagonist, in a rat model of postoperative pain. Eur J Pharmacol 344:115–120

Gonzalez MI, Field MJ, Hughes J, Singh L (2000) Evaluation of selective NK_1 receptor antagonist CI-1021 in animal models of inflammatory and neuropathic pain. J Pharmacol Exp Ther 294:444–450

Guard S, Watson SP (1991) Tachykinin receptor types: classification and membrane signalling mechanisms. Neurochem Int 18:149–165

Guard S, Boyle SJ, Tang KW, Watling KJ, McKnight AT, Woodruff GN (1993) The interaction of the NK_1 receptor antagonist CP-96,345 with L-type calcium channels and its functional consequences. Br J Pharmacol 110:385–391

Guo JZ, Yoshioka K, Zhao FY, Hosoki R, Maehara T, Yanagisawa M, Hagan RM, Otsuka M (1995) Pharmacological characterization of GR82334, a tachykinin NK_1 receptor antagonist, in the isolated spinal cord of the neonatal rat. Eur J Pharmacol 281:49–54

Hagan RM, Ireland SJ, Jordan CC, Beresford IJM, Stephens-Smith ML, Ewan G, Ward P (1990) GR 71251, a novel, potent and highly selective antagonist at neurokinin NK-1 receptors. Br J Pharmacol 99:62P

Hagan RM, Ireland SJ Bailey P, Mcbride C, Jordan CC, Ward, P (1991) A spirolactam conformationally-constrained analogue of physalaemin which is a peptidase-resistant selective neurokinin NK_1 receptor antagonist. Br J Pharmacol 102:168P

Hagiwara D, Miyake H, Igari N, Murano K, Morimoto H, Murai M, Fujii T, Matsuo M (1993) Design of a novel dipeptide substance P antagonist FK888. Regul Pept 46:332–334

Hale JJ, Mills SG, MacCoss M, Shah SK, Qi H, Mathre DJ, Cascieri MA, Sadowski S, Strader CD, MacIntyre DE, Metzger JM (1996) 2(S)-((3,5-bis(trifluoromethyl)benzyl)-oxy)-3(S)-phenyl-4- ((3-oxo-1,2,4-triazol-5-yl)methyl)morpholine (1): a potent, orally active, morpholine-based human neurokinin-1 receptor antagonist. J Med Chem 39:1760–1762

Hale JJ, Mills SG, MacCoss M, Finke PE, Cascieri MA, Sadowski S, Ber E, Chicchi GG, Kurtz M, Metzger J, Eiermann G, Tsou NN, Tattersall FD, Rupniak NM, Williams AR, Rycroft W, Hargreaves R, MacIntyre DE (1998) Structural optimization affording 2-(R)-(1-(R)-3, 5-bis(trifluoromethyl)phenylethoxy)-3-(S)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine, a potent, orally active, long-acting morpholine acetal human NK-1 receptor antagonist. J Med Chem 41:4607–4614

Hale JJ, Mills SG, MacCoss M, Dorn CP, Finke PE, Budhu RJ, Reamer RA, Huskey SE, Luffer-Atlas D, Dean BJ, McGowan EM, Feeney WP, Chiu SH, Cascieri MA, Chicchi GG, Kurtz MM, Sadowski S, Ber E, Tattersall FD, Rupniak NM, Williams AR, Rycroft W, Hargreaves R, Metzger JM, MacIntyre DE (2000) Phosphorylated morpholine acetal human neurokinin-1 receptor antagonists as water-soluble prodrugs. J Med Chem 43:1234–1241

Hall JM, Mitchell D, Morton IK (1994) Typical and atypical NK_1 tachykinin receptor characteristics in the rabbit isolated iris sphincter. Br J Pharmacol 112:985–991

Harrison T, Williams BJ, Swain CJ, Ball RG (1994) Piperidine-ether based hNK_1 antagonists 1: determination of the relative and absolute stereochemical requirements. Biomed Chem Lett 4:2545–2550

Hastrup H, Schwartz TW (1996) Septide and neurokinin A are high affinity ligands on the NK_1 receptor:evidence from homologus versus heterologus binding analysis. FEBS Lett 399:264–266

Hauser K, Heid J, Criscione L, Brugger F, Ofner S, Veenstra S, Schilling W (1994) CGRP 49,823, a novel, nonpeptidic NK_1 receptor antagonist : in vitro pharmacology. Neuropeptides 26:37

Hesketh PJ, Gralla RJ, Webb RT, Ueno W, DelPrete S, Bachinsky ME, Dirlam NL, Stack CB, Silberman SL (1999) Randomized phase II study of the neurokinin 1 receptor antagonist CJ-11,974 in the control of cisplatin-induced emesis. J Clin Oncol 17:338–343

Hill R (2000a) NK_1 (substance P) receptor antagonists—why are they not analgesic in humans? Trends Pharmacol Sci 21:244–246

Hill R (2000b) Reply: will changing the testing paradigms show that NK_1 receptor antagonists are analgesic in humans? Trends Pharmacol Sci 21:465

Hipskind PA, Howbert JJ, Bruns RF, Cho SS, Crowell TA, Foreman MM, Gehlert DR, Iyengar S, Johnson KW, Krushinski JH, Li DL, Lobb KL, Mason NR, Muehl BS, Nixon JA, Phebus LA, Regoli D, Simmons RM, Threlkeld PG, Waters DC, Gitter BD (1996) 3-Aryl-1,2-diacetamidopropane derivatives as novel and potent NK-1 receptor antagonists. J Med Chem 39:736–748

Hirayama Y, Lei YH, Barnes PJ, Rogers DF (1993) Effects of two novel tachykinin antagonists, FK224 and FK888, on neurogenic airway plasma exudation, bronchoconstriction and systemic hypotension in guinea-pigs in vivo. Br J Pharmacol 108:844–851

Hökfelt T, Vincent S, Hellsten L, Rosell S, Folkers K, Markey K, Goldstein M, Cuello C (1981) Immunohistochemical evidence for a 'neurotoxic' action of (D-Pro^2, D-$Trp^{7,9}$)-substance P, an analogue with substance P antagonistic activity. Acta Physiol Scand 113:571–573

Holzer P (1998) Tachykinins as targets of gastroenterological pharmacotherapy. Drugs News Perspect 11:394–401

Holzer P, Holzer-Petsche U (1997) Tachykinins in the gut Part II Roles in neural excitation, secretion and inflammation. Pharmacol Ther 73:219–263

Holzer-Petsche U, Rordorf-Nikolic T (1995) Central versus peripheral site of action of the tachykinin NK_1-antagonist RP 67580 in inhibiting chemonociception. Br J Pharmacol 115:486–490

Hoover DB (1991) Effects of spantide on guinea-pig coronary resistance vessels. Peptides 12:983–988

Huang R-RC, Yu H, Strader CD, Fong TM (1994) Interaction of substance P with the second and seventh transmembrane domain of the NK_1 receptor. Biochemistry 33:3007–3013

Ichinose M, Miura M, Yamauchi H, Kageyama N, Tomaki M, Oyake T, Ohuchi Y, Hida W, Miki H, Tamura G, Shirato K (1996) A neurokinin 1-receptor antagonist improves exercise-induced airway narrowing in asthmatic patients. Am J Resp Crit Care Med 153:936–941

Inoue H, Nagata N, Koshihara Y (1996) Effect of the tachykinin receptor antagonists, SR 140333, FK 888, and SR 142801, on capsaicin-induced mouse ear oedema. Inflamm Res 45:303–307

Iyengar S, Hipskind PA, Gehlert DR, Schober D, Lobb KL, Nixon JA, Helton DR, Kallman MJ, Boucher S, Couture R, Li DL, Simmons RM (1997) LY303870, a centrally active neurokinin-1 antagonist with a long duration of action. J Pharmacol Exp Ther 280:774–785

Jenkinson KM, Southwell BR, Furness JB (1999) Two affinities for a single antagonist at the neuronal NK_1 tachykinin receptor: evidence from quantitation of receptor endocytosis. Br J Pharmacol 126:131–136

Jung M, Calassi R, Maruani J, Barnouin MC, Souilhac J, Poncelet M, Gueudet C, Emonds-Alt X, Soubrie P, Breliere JC (1994) Neuropharmacological characterization of SR 140333, a non peptide antagonist of NK_1 receptors. Neuropharmacology 33:167–179

Karlsson U, Nasstrom J, Berge OG (1994) (+/-)-CP-96,345, an NK_1 receptor antagonist, has local anaesthetic-like effects in a mammalian sciatic nerve preparation. Regul Pept 52:39–46

Khan S, Liu YC, Khawaja AM, Manzini S, Rogers DF (2001) Effect of the long-acting tachykinin NK_1 receptor antagonist MEN 11467 on tracheal mucus secretion in allergic ferrets. Br J Pharmacol 132:189–196

Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, Reines SA, Liu G, Snavely D, Wyatt-Knowles E, Hale JJ, Mills SG, MacCoss M, Swain CJ, Harrison T, Hill RG, Hefti F, Scolnick EM, Cascieri MA, Chicchi GG, Sadowski S, Williams AR, Hewson L, Smith D, Carlson EJ, Hargreaves RJ, Rupniak NM (1998) Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 281:1640–1645

Kris MG, Radford JE, Pizzo BA, Inabinet R, Hesketh A, Hesketh PJ (1997) Use of an NK_1 receptor antagonist to prevent delayed emesis after cisplatin. J Natl Cancer Inst 89:817–818

Lei YH, Barnes PJ, Rogers DF (1992) Inhibition of neurogenic plasma exudation in guinea-pig airways by CP-96,345, a new non-peptide NK_1 receptor antagonist. Br J Pharmacol 105:261–262

Leander S, Hakanson R, Rosell S, Folkers K, Sundler F, Tornqvist K (1981) A specific SP antagonist blocks smooth muscle contractions induced by noncholinergic nonadrenergic nerve stimulation. Nature 294:467–469

Lecci A, Giuliani S, Patacchini R, Viti G, Maggi CA (1991) Role of NK_1 tachykinin receptors in thermonociception: effect of (±)- CP 96,345, a non peptide substance P antagonist on the hot plate test in mice. Neurosci Letters 129:299–302

Lee OY, Munakata J, Naliboff, BD, Chang L, Mayer E (2000) A double blind parallel group pilot study of the effects of CJ-11,974 and placebo on perceptual and emotional responses to rectosigmoid distension in IBS patients. Gastroenterology 118:4439

Lee WS, Moussaoui SM, Moskowitz MA (1994) Blockade by oral or parenteral RPR 100893 (a non-peptide NK_1 receptor antagonist) of neurogenic plasma protein extravasation within guinea-pig dura mater and conjunctiva. Br J Pharmacol 112:920–924

Lembeck F, Donnerer J, Tsuchiya M, Nagahisa A (1992) The non-peptide tachykinin antagonist, CP-96,345, is a potent inhibitor of neurogenic inflammation. Br J Pharmacol 105:527–530

Lombet A, Spedding M (1994) Differential effects of nonpeptidic tachykinin receptor antagonists on Ca2+ channels. Eur J Pharmacol 267:113–115

Longmore J, Razzaque Z, Shaw D, Hill RG (1994) Differences in the effects of NK_1-receptor antagonists, (+/-)-CP 96,345 and CP 99,994, on agonist-induced responses in guinea-pig trachea. Br J Pharmacol 112:176–178

Lowe JA 3rd, Drozda SE, Snider RM, Longo KP, Zorn SH, Morrone J, Jackson ER, McLean S, Bryce DK, Bordner J (1992) The discovery of (2S,3S)-cis-2-(diphenylmethyl)-N-[(2-methoxyphenyl)methyl]-1- azabicyclo[222]-octan-3-amine as a novel, nonpeptide substance P antagonist. J Med Chem 35:2591–2600

Lucot JB, Obach RS, McLean S, Watson JW (1997) The effect of CP-99994 on the responses to provocative motion in the cat. Br J Pharmacol 120:116–120

MacLeod AM, Merchant KJ, Brookfield F, Kelleher F, Stevenson G, Owens AP, Swain CJ, Casiceri MA, Sadowski S, Ber E (1994) Identification of L-tryptophan derivatives with potent and selective antagonist activity at the NK_1 receptor. J Med Chem 37:1269–1274

MacLeod AM, Cascieri MA, Merchant KJ, Sadowski S, Hardwicke S, Lewis RT, MacIntyre DE, Metzger JM, Fong TM, Shepheard S (1995) Synthesis and biological evaluation of NK_1 antagonists derived from L-tryptophan. J Med Chem 38:934–941

Maggi CA (1995) The mammalian tachykinin receptors. Gen Pharmacol 26:911–944

Maggi CA, Schwartz TW (1997) The dual nature of the tachykinin NK_1 receptor. Trends Pharmacol Sci 18:351–355

Maggi CA, Patacchini R, Feng DM, Folkers K (1991) Activity of spantide I and II at various tachykinin receptors and NK-2 tachykinin receptor subtypes. Eur J Pharmacol 199:127–129

Maggi CA, Giuliani S, Patacchini R, Santicioli P, Theodorsson E, Barbanti G, Turini D, Giachetti A (1992) Tachykinin antagonists inhibit nerve-mediated contractions in the circular muscle of the human ileum. Gastroenterology 102:88–96

Maggi CA, Patacchini R, Meini S, Giuliani S (1993a) Evidence for the presence of a septide-sensitive tachykinin receptor in the circular muscle of the guinea-pig ileum. Eur J Pharmacol 235:309–311

Maggi CA, Patacchini R, Rovero P, Giachetti A (1993b) Tachykinin receptors and tachykinin receptor subtypes. J Autonom Pharmacol 13:23–93

Maggi CA, Patacchini R, Meini S, Quartara L, Sisto A, Potier E, Giuliani S, Giachetti A (1994) Comparison of tachykinin NK_1 and NK_2 receptors in the circular muscle of the guinea-pig ileum and proximal colon. Br J Pharmacol 112:150–160

Mazelin L, Theodorou V, More J, Emonds-Alt X, Fioramonti J and Bueno L (1998) Comparative effects onf nonpeptide tachykinin receptor antagonists in experimental gut inflammation in rats and guinea-pigs. Life Sci 63:293–304

McAllister KH, Pratt JA (1998) GR205171 blocks apomorphine and amphetamine-induced conditioned taste aversions Eur J Pharmacol 353:141–148

McKnight AT, Boyle SJ, Tang K-W, Hill DR, Sauman-Chauhan N, Woodruff GN (1994) Functional activity at NK_1 receptors of novel peptoid antagonists. Br J Pharmacol 111:49P

McLean S, Ganong A, Seymour PA, Snider RM, Desai MC, Rosen T, Bryce DK, Longo KP, Reynolds LS, Robinson G, Schmidt AW, Siok C, Heym J (1993) Pharmacology of CP-99,994; a nonpeptide antagonist of the tachykinin neurokinin-1 receptor. J Pharmacol Exp Ther 267:472–479

McLean S, Ganong A, Seymour PA, Bryce DK, Crawford RT, Morrone J, Reynolds LS, Schmidt AW, Zorn S, Watson J, Fossa A, DePasquale M, Rosen T, Nagahisa A, Tsuchiya M, Heym J (1996) Characterization of CP-122,721; a nonpeptide antagonist of the neurokinin NK_1 receptor. J Pharmacol Exp Ther 277:900–908

Megens AA, Ashton D, Vermeire JC, Vermote PC, Hens KA, Hillen LC, Fransen JF, Mahieu M, Heylen L, Leysen JE, Jurzak MR, Janssens F (2002) Pharmacological profile of (2R-trans)-4-[1-[3,5-bis(trifluoromethyl)benzoyl]-2-(phenylmethyl)-4-piperidinyl]-N-(2,6-dimethylphenyl)-1-acetamide (S)-Hydroxybutanedioate (R116301), an orally and centrally active neurokinin-1 receptor antagonist. J Pharmacol Exp Ther 302:696–709

Meini S, Patacchini R, Maggi CA (1994) Tachykinin NK_1 receptor subtypes in the rat urinary bladder. Br J Pharmacol 111:739–746

Minami M, Endo T, Kikuchi K, Ihira E, Hirafuji M, Hamaue N, Monma Y, Sakurada T, Tan-no K, Kisara K (1998) Antiemetic effects of sendide, a peptide tachykinin NK_1 receptor antagonist, in the ferret. Eur J Pharmacol 363:49–55

Montier F, Carruette A, Moussaoui S, Boccio D, Garret C (1994) Antagonism of substance P and related peptides by RP 67580 and CP-96,345, at tachykinin NK_1 receptor sites, in the rat urinary bladder. Eur J Pharmacol 251:9–14

Moriarty D, Goldhill J, Selve N, O'Donoghue DP, Baird AW (2001) Human colonic antisecretory activity of the potent NK_1 antagonist, SR140333: assessment of potential anti-diarrhoeal activity in food allergy and inflammatory bowel disease. Br J Pharmacol 133:1346–1354

Morimoto H, Murai M, Maeda Y, Hagiwara D, Miyake H, Matsuo M, Fujii T (1992) FR 113680: a novel tripeptide substance P antagonist with NK_1 receptor selectivity. Br J Pharmacol 106:123–126

Moussaoui SM, Montier F, Carruette A, Blanchard JC, Laduron PM, Garret C A (1993) non-peptide NK_1-receptor antagonist, RP 67580, inhibits neurogenic inflammation postsynaptically. Br J Pharmacol 109:259–264

Moussaoui SM, Montier F, Carruette A, Fardin V, Floch A, Garret C (1994) In vivo pharmacological properties of RPR 100893, a novel non-peptide antagonist of the human NK_1 receptor. Neuropeptides 26:35

Murai M, Morimoto H, Maeda Y, Fujii T (1992) Effects of the tripeptide substance P antagonist, FR113680, on airway constriction and airway edema induced by neurokinins in guinea-pigs. Eur J Pharmacol 217:23–29

Murai M, Maeda Y, Hagiwara D, Miyake H, Ikari N, Matsuo M, Fujii T (1993) Effects of an NK_1 receptor antagonist, FK888, on constriction and plasma extravasation induced in guinea-pig airway by neurokinins and capsaicin. Eur J Pharmacol 236:7–13

Mussap CJ, Geraghty DP, Burcher E (1993) Tachykinin receptors: a radioligand binding perspective. J Neurochem 6:1987–2009

Nagahisa A, Kanai Y, Suga O, Taniguchi K, Tsuchiya M, Lowe JA 3rd, Hess HJ (1992) Antiinflammatory and analgesic activity of a non-peptide substance P receptor antagonist. Eur J Pharmacol 217:191–195

Natsugari H, Ikeura Y, Kiyota Y, Ishichi Y, Ishimaru T, Saga O, Shirafuji H, Tanaka T, Kamo I, Doi T (1995) Novel, potent, and orally active substance P antagonists: synthesis and antagonist activity of N-benzylcarboxamide derivatives of pyrido[3,4-b]pyridine. J Med Chem 38:3106–3120

Natsugari H, Ikeura Y, Kamo I, Ishimaru T, Ishichi Y, Fujishima A, Tanaka T, Kasahara F, Kawada M, Doi T (1999) Axially chiral 1,7-naphthyridine-6-carboxamide derivatives as orally active tachykinin NK_1 receptor antagonists: synthesis, antagonistic activity, and effects on bladder functions. J Med Chem 42:3982–3993

Navari RM, Reinhardt RR, Gralla RJ, Kris MG, Hesketh PJ, Khojasteh A, Kindler H, Grote TH, Pendergrass K, Grunberg SM, Carides AD, Gertz BJ (1999) Reduction of cisplatin-induced emesis by a selective neurokinin-1-receptor antagonist. New Engl J Med 340:190–195

Okano S, Nagaya H, Ikeura Y, Natsugari H, Inatomi N (2001) Effects of TAK-637, a novel neurokinin-1 receptor antagonist, on colonic function in vivo. J Pharmacol Exp Ther 298:559–564

Okano S, Ikeura Y, Inatomi N (2002) Effects of tachykinin NK_1 receptor antagonists on the viscerosensory response caused by colorectal distention in rabbits. J Pharmacol Exp Ther 300:925–931

Palma C, Goso C, Manzini S (1994) Different susceptibility to neurokinin 1 receptor antagonists of substance P and septide-induced interleukin-6 release from U373 MG human astrocytoma cell line. Neurosci Lett 171:221–224

Papp M, Vassout A, Gentsch C (2000) The NK_1-receptor antagonist NKP608 has an antidepressant-like effect in the chronic mild stress model of depression in rats. Behav Brain Res 115:19–23

Patacchini R, Maggi CA (1995) Tachykinin NK_1 receptors mediate both vasoconstrictor and vasodilator responses in the rabbit isolated jugular vein. Eur J Pharmacol 283:233–240

Patacchini R, Santicioli P, Astolfi M, Rovero P, Viti G & Maggi CA (1992) Activity of peptide and non-peptide antagonists at peripheral NK-1 tachykinin receptors. Eur J Pharmacol 215:93–98

Patacchini R, Quartara L, Astolfi M, Goso C, Giachetti A, Maggi CA (1995) Activity of cyclic pseudopeptide antagonists at peripheral tachykinin receptors. J Pharmacol Exp Ther 272:1082–1087

Patacchini R, Bartho L, Maggi CA (1997) Characterization of receptors mediating contraction induced by tachykinins in the guinea-pig isolated common bile duct. Br J Pharmacol 122:1633–1638

Petitet F, Saffroy M, Torrens Y, Lavielle S, Chassaing G, Loeuillet D, Glowinski J & Beaujouan JC (1992) Possible existence of a new tachykinin receptor subtype in the guinea-pig ileum. Peptides 13:383–388

Phebus LA, Johnson KW, Stengel PW, Lobb KL, Nixon JA, Hipskind PA (1997) The non-peptide NK-1 receptor antagonist LY303870 inhibits neurogenic dural inflammation in guinea-pigs. Life Sci 60:1553–1561

Piedimonte G, Bertrand C, Geppetti P, Snider RM, Desai MC, Nadel JA (1993) A new NK_1 receptor antagonist (CP-99,994) prevents the increase in tracheal vascular permeability produced by hypertonic saline. J Pharmacol Exp Ther 266:270–273

Pradier L, Menager J, Le Guern J, Bock MD, Heuillet E, Fardin V, Garret C, Doble A, Mayaux JF (1994) Septide: an agonist for the NK_1 receptor acting at a site distinct from substance P. Mol Pharmacol 45:287–93

Quartara L, Maggi CA (1997) The tachykinin NK-1 receptor. Part I: ligands and mechanisms of cellular activation. Neuropeptides 31:537–553

Quartara L, Maggi CA (1998) The tachykinin NK-1 receptor. Part II: distribution and pathopysiological roles. Neuropeptides 32:1–49

Radhakrishnan V, Henry JL (1991) Novel substance P antagonist, CP-96,345, blocks responses of cat spinal dorsal horn neurons to noxious cutaneous stimulation and to substance P. Neurosci Lett 132:39–43

Regoli D, Escher E, Drapeau G, D'Orleans-Juste P, Mizrahi J (1984) Receptors for substance P III Classification by competitive antagonists. Eur J Pharmacol 97:179–189

Regoli D, Drapeau G, Dion S, D'Orleans-Juste P (1989) Receptors for substance P and related neurokinins. Pharmacology 38:1–15

Regoli D, Boudon A and Fauchere J (1994) Receptors and antagonists for substance P and related peptides. Pharmacol Rev 46:551–599

Reid K, Palmer JL, Wright RJ, Clemes SA, Troakes C, Somal HS, House B, Stott JR (2000) Comparison of the neurokinin-1 antagonist GR205171, alone and in combination with the 5-HT3 antagonist ondansetron, hyoscine and placebo in the prevention of motion-induced nausea in man. Br J Clin Pharmacol 50:61–64

Romerio SC, Linder L, Haefeli WE (1999) Neurokinin-1 receptor antagonist R116301 inhibits substance P-induced venodilation. Clin Pharmacol Ther 66:522–527

Rupniak NMJ, Kramer MS (1999) Discovery of the anti-depressant and anti-emetic efficacy of substance P receptor (NK_1) antagonists. Trends Pharmacol Sci 20:485–489

Rupniak NM, Boyce S, Williams AR, Cook G, Longmore J, Seabrook GR, Caeser M, Iversen SD, Hill RG (1993) Antinociceptive activity of NK_1 receptor antagonists: non-specific effects of racemic RP67580. Br J Pharmacol 110:1607–1613

Rupniak NM, Webb JK, Williams AR, Carlson E, Boyce S, Hill RG (1995) Antinociceptive activity of the tachykinin NK_1 receptor antagonist, CP-99,994, in conscious gerbils. Br J Pharmacol 116:1937–1943

Rupniak NM, Carlson E, Boyce S, Webb JK, Hill RG (1996) Enantioselective inhibition of the formalin paw late phase by the NK_1 receptor antagonist L-733,060 in gerbils. Pain 67:189–195

Rupniak NM, Tattersall FD, Williams AR, Rycroft W, Carlson EJ, Cascieri MA, Sadowski S, Ber E, Hale JJ, Mills SG, MacCoss M, Seward E, Huscroft I, Owen S, Swain CJ, Hill RG, Hargreaves RJ (1997) In vitro and in vivo predictors of the anti-emetic activity of tachykinin NK_1 receptor antagonists. Eur J Pharmacol 326:201–209

Sachais BS, Krause JE (1994) Both extracellular and transmembrane residues contribute to the species selectivity of the neurokinin-1receptor antagonist WIN 51708. Mol Pharmacol 46:122–128

Sachais BS, Snider RM, Lowe JA 3rd, Krause JE (1993) Molecular basis for the species selectivity of the substance P antagonist CP-96,345. J Biol Chem 268:2319–23

Sakurada T, Manome Y, Tan-No K, Sakurada S, Kisara K, Ohba M, Terenius L A (1992) Selective and extremely potent antagonist of the neurokinin-1 receptor. Brain Res 593:319–322

Sakurada T, Katsumata K, Yogo H, Tan-No K, Sakurada S, Kisara K (1993) Antinociception induced by CP 96,345, a non-peptide NK-1 receptor antagonist, in the mouse formalin and capsaicin tests. Neurosci Lett 151:142–145

Sakurada T, Katsumata K, Yogo H, Tan-No K, Sakurada S, Ohba M, Kisara K (1995) The neurokinin-1 receptor antagonist, sendide, exhibits antinociceptive activity in the formalin test. Pain 60:175–180

Schmidt AW, McLean S, Heym J (1992) The substance P receptor antagonist CP-96,345 interacts with Ca^{2+} channels. Eur J Pharmacol 219:491–492

Schwartz TW, Gether U, Schambye HT, Hjorth SA (1995) Molecular mechanisms of action of nonpeptide ligands for peptide receptors. Curr Pharmac Design 1:325–342

Seabrook GR, Shepheard SL, Williamson DJ, Tyrer P, Rigby M, Cascieri MA, Harrison T, Hargreaves RJ, Hill RG (1996) L-733,060, a novel tachykinin NK_1 receptor antagonist; effects in [Ca2+]i mobilisation, cardiovascular and dural extravasation assays. Eur J Pharmacol 317:129–135

Shepheard SL, Williamson DJ, Hill RG, Hargreaves RJ (1993) The non-peptide neurokinin1 receptor antagonist, RP 67580, blocks neurogenic plasma extravasation in the dura mater of rats. Br J Pharmacol 108:11–12

Singh L, Field MJ, Hughes J, Kuo BS, Suman-Chauhan N, Tuladhar BR, Wright DS, Naylor RJ (1997) The tachykinin NK_1 receptor antagonist PD 154075 blocks cisplatin-induced delayed emesis in the ferret. Eur J Pharmacol 321:209–216

Smith G, Harrison S, Bowers J, Wiseman J, Birch P (1994) Non-specific effects of the tachykinin NK_1 receptor antagonist, CP-99,994, in antinociceptive tests in rat, mouse and gerbil. Eur J Pharmacol 271:481–487

Snider RM, Constantine JW, Lowe III JA, Longo KP, Lebel WS, Woody HA, Drozda SE, Desai MC, Vinick FJ, Spencer RW Hess HJ (1991) A potent nonpeptide antagonist of the SP (NK-1) receptor. Science 251:435–437

Sonea IM, Palmer MV, Akili D, Harp JA (2002) Treatment with neurokinin-1 receptor antagonist reduces severity of inflammatory bowel disease induced by Cryptosporidium parvum. Clin Diagn Lab Immunol 9:333–340

Steinberg R, Alonso R, Rouquier L, Desvignes C, Michaud JC, Gudennec A, Jung M, Simiand J, Griebel G, Emonds-Alt X, Le Fur G, Soubrie P (2002) SSR240600 [(R)-2-(1-[2-[4-[2-[3,5-bis(trifluoromethyl)phenyl]acetyl]-2-(3,4-dichlorophenyl)-2-morpholinyl]ethyl]-4-piperidinyl)-2-methylpropanamide], a centrally active nonpeptide antagonist of the tachykinin neurokinin 1 receptor: II Neurochemical and behavioral characterization. J Pharmacol Exp Ther 303:1180–1188

Stucchi AF, Shofer S, Leeman S, Materne O, Beer E, McClung J, Shebani K, Moore F, O'Brien M, Becker JM (2000) NK-1 antagonist reduces colonic inflammation and oxidative stress in dextran sulfate-induced colitis in rats. Am J Physiol 279:G1298–G1306

Suarez GA, Opfer-Gehrking TL, McLean DB (1994) Double-blind, placebo-controlled study of the efficacy of a substance P (NK_1) receptor antagonist in painful peripheral neuropathy. Neurology 44:A220

Swain CJ, Seward EM, Sabin V, Cascieri MA (1993) Quinuclidine based NK-1 antagonists 2: determination of the absolute stereochemical requirements. Biomed Chem Lett 3:1703–1706

Tamura K, Mutabagani K, Wood JD (1993) Analysis of a nonpeptide antagonist for substance P on myenteric neurons of guinea-pig small intestine. Eur J Pharmacol 232:235–239

Tattersall FD, Rycroft W, Hargreaves RJ, Hill RG (1993) The tachykinin NK_1 receptor antagonist CP-99,994 attenuates cisplatin induced emesis in the ferret. Eur J Pharmacol 250:R5–R6

Tattersall FD, Rycroft W, Marmont N, Cascieri M, Hill RG, Hargreaves RJ (1995) Enantiospecific inhibition of emesis induced by nicotine in the house musk shrew (Suncus murinus) by the neurokinin 1 (NK_1) receptor antagonist CP-99,994. Neuropharmacology 34:1697–1699